Dispersal of plants in the Central European landscape – dispersal processes and assessment of dispersal potential exemplified for endozoochory

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Contents

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Chapter 1 General introduction

As early as 1873 HILDEBRAND realized that dispersal is one of the fundamental processes in the life cycle of each plant. Dispersal is important to escape from the parent plant in order to avoid intraspecific competition as well as predation by animals which is density dependent and therefore highest in the vicinity of the parent plant (HOWE & SMALLWOOD 1982; DIRZO & DOMINGUEZ 1986). It enables species to (re-)colonize unoccupied habitats and is therefore a limiting factor within the dynamics of metapopulations (HUSBAND & BARRETT 1996; POSCHLOD 1996; CAIN et al. 2000). Furthermore, dispersal affects the level of gene flow (YOUNG et al. 1996) and therefore influences processes as local adaptation or speciation (BARTON & HEWITT 1989; HARRISON & HASTINGS 1996). According to the ”species pool-concept”, dispersal is a major factor controlling the composition and species richness of plant communities (PÄRTEL et al. 1996; ZOBEL 1997; ZOBEL et al. 1998) and may therefore limit species richness, diversity and dynamics (see also CAIN et al. 2000). It is related to coexistence of plant species within communities (LEVIN 1974), because dispersal is a driving factor in the carousel model (VAN DER MAAREL & SYKES 1993). Hence, it is difficult to imagine any ecological or evolutionary question that is not affected by dispersal (DIECKMANN et al. 1999). Each discussion concerning the dynamics of plant populations should therefore consider dispersal ecological parameters (HARPER 1977; BONN & POSCHLOD 1998a).

Over decades, the occurrence of plant species was, however, merely attributed to various biotic and abiotic factors, such as nutrient and water supply, light regime or intensity of disturbance (e.g. ELLENBERG 1996). Changes in species composition of plant communities as well as the dramatic decrease and endangerment of numerous plant species during the last decades were usually interpreted as a mere result of environmental changes, the decline of habitat quality by intensification, eutrophication, abandonment a.o. or the complete loss of habitats (KORNECK & SUKOPP 1988). Until recently (POSCHLOD 1996; POSCHLOD et al. 1997, 1998; BONN & POSCHLOD 1998a,b; POSCHLOD & BONN 1998) dispersability of plant species remained disregarded in this context, although numerous extensive comparative text books concerning the dispersal of plant species have been written (SERNANDER 1906; RIDLEY 1930; MÜLLER-SCHNEIDER 1977; LUFTENSTEINER 1982; VAN DER PIJL 1982; MURRAY 1986). The more or less continuous research concerning the dispersal of plants, as indicated by these publications, on the one hand and the lacking consideration of dispersal ecology with respect to species composition and richness of plant communities on the other hand may be explained by several deficits:

- most of the existing text books concerning diaspore dispersal were restricted in documenting single dispersal events or in classifying the different modes of dispersal, allocating species to only one of the different dispersal modes, although diaspores are usually polychorous, which

means that they can be dispersed by different vectors (e.g. DANSEREAU & LEMS 1957; LIDDLE & ELGAR 1984; JOHANSSON & NILSSON 1996);

- in most studies, the dispersal mode of a certain species was merely deduced from diaspore morphology, although as early as 1928 SIMEON concluded his thesis on 'seed formation and dispersal' with the following sentences: "Undoubtedly, many questions on the ecology of seed formation and seed dispersal, particularly regarding the efficacy of the different means of dispersal remain yet to be answered. Today, specific [morphological] characteristics of fruits and seeds are regarded as such means. But do they indeed serve this purpose? Do they really ensure the dispersal of the respective plants to new habitats? Investigating this seems to me the task of future dispersal ecology."*;
- species were classified as either dispersed or not dispersed, although dispersal potential by a certain vector is gradual, varying from very high to very low dispersal potential (TACKENBERG 2001; TACKENBERG et al. 2003);
- none of these works related dispersal to the actual distribution of plant species;
- the manifold dispersal processes in our cultural landscape were not considered, although nearly all Central European plant communities are man-made or at least modified by man (BONN & POSCHLOD 1998a,b; POSCHLOD & BONN 1998).

Due to these deficits, a consideration of this "classical" knowledge concerning species dispersability was little helpful for an interpretation of the composition of plant communities or for the risk assessment of plant species. Furthermore, the limited usefulness of dispersal data was manifested by studies documenting the diaspore input in different plant communities by catching the diaspore rain with diaspore traps (funnels). These studies concluded that the majority of the diaspores caught is dispersed only over short distances and that the diaspore rain merely reflects the species composition of the surrounding vegetation (LUFTENSTEINER 1982; VERKAAR et al. 1983; FISCHER 1987; ROBINSON & QUINN 1988; PEART 1989a,b; POSCHLOD & JORDAN 1996; POSCHLOD & JACKEL 1993; POSCHLOD et al. 1996b). On the one hand, however, certain dispersal modes relevant for long distance dispersal (e.g. zoochory) could hardly be measured by this method. On the other hand, rare long-distance dispersal events which influence many key aspects of the biology of plants, such as metapopulation dynamics or diversity and dynamics in plant communities, are nearly impossible to be exactly measured anyway (SILVERTOWN & LOVETT-DOUST 1993; BULLOCK & CLARKE 2000; CAIN et al. 2000).

* Original text: „Unzweifelhaft gibt es in der Oekologie der Samenbildung und Samenverbreitung noch viele Fragen, die einer Beantwortung harren. Dies bezieht sich vor allem auf die Wirksamkeit der Verbreitungsmittel. Man sieht wohl heute verschiedene Einrichtungen der Früchte und Samen als solche an. Aber sind sie es in Wirklichkeit? Sorgen sie tatsächlich für eine Ausbreitung der betreffenden Pflanzen an neue Standorte? Darüber Klarheit zu schaffen, scheint mir die Aufgabe der Verbreitungsökologie der Zukunft ...".

The massive change of our cultural landscape, accompanied by an increasing fragmentation and isolation of habitats recently led, however, to a critical reflection in plant ecology and the risk assessment for plant species. For fragmented populations, dispersal now was called a “key to survival“ (OPDAM 1990). Recently, BONN & POSCHLOD (1998a,b) and POSCHLOD & BONN (1998) reviewed the big variety of processes existing in natural, traditional and actual man-made landscapes which once were or are at present important for the dispersal of plant species (see also chap. 2). The change of these processes was shown to be an important reason for the lacking reestablishment of plant species in restored habitats (e.g. POSCHLOD et al. 1997, 1998). In our present cultural landscape, dispersal therefore proved to be a limiting factor in restoration management and the recreation of species rich communities (e.g. VAN GROENENDAEL et al. 1989; KAPFER 1996; KLEINSCHMIDT & ROSENTHAL 1995; KOWARSCH et al. 2001; PYWELL et al. 2002).

However, a risk assessment for plant species in the context of conservation biology and restoration ecology requires not only the knowledge concerning changes or losses of dispersal processes in our landscape but also adequate methods or “tools” to predict dispersability of a certain plant species by various dispersal vectors in order to detect dispersal limitations or to model dispersal processes at the landscape level (POSCHLOD et al. 2000; TACKENBERG 2001, 2003; TACKENBERG et al. 2003; HIGGINS et al. 2003). Such methods or tools aiming at a quantification of a species dispersal potential may be developed by different approaches:

- by a data base including existing data on documented dispersal modes and distances as well as dispersal related diaspore and plant traits in (BONN et al. 2000);
- by deriving the dispersal potential from dispersal relevant diaspore and plant attributes (e.g. TACKENBERG et al. 2003);
- by the assessment of dispersal via standardized methodological approaches (e.g. OCUMPAUGH & SWAKON 1993).

In the following chapters, which also can be read separately, dispersal processes in a changing landscape as well as the mentioned approaches concerning a quantification of the dispersal potential of plant species will be highlighted:

- in chapter 2, which is based on BONN & POSCHLOD (1998a,b) and POSCHLOD & BONN (1998), dispersal-relevant processes existing in the natural, traditional and actual man-made landscape of Central Europe are analysed;
- in chapter 3, the conception of a database (»DIASPORUS«) containing standardised species related traits relevant for seed or diaspore dispersal and documented dispersal modes for single plant species is illustrated (see also BONN et al. 2000);
- in chapter 4 and 5, two different approaches for an assessment of species dispersal potential are presented, exemplified for endozoochorous dispersal by ruminants:

- in chapter 4, various methodological approaches are tested for their suitability for a standardized assessment of endozoochorous dispersal potential.
- in chapter 5, the possibilities of a deduction of endozoochorous dispersal from various diaspore and plant attributes and site-specific variables, respectively, are illustrated.

Chapter 2 Dispersal processes in the Central European landscape in the change of time – an explanation for the present decrease of plant species diversity in different habitats?

Abstract

During the evolution of the Central European landscape and especially since the settlement of man there was a permanent change of processes affecting dispersability of plants. The highest diversity of dispersal processes combined with a high diversity of agricultural land use practices existed in the traditional man-made landscape. In the actual man-made landscape most of these processes changed or became completely lost.

Due to the improvement of seed cleaning a lot of weed species became extinct which were spread in former times with contaminated seed. Changing harvest methods were responsible for the selection of weeds which ripe later and produce light diaspores. Traditional manure contained huge amounts of diaspores whereas today animal slurry with low diaspore contents or mineral fertilizer are used. Artificial flooding practices favoured the migration of species in meadows of mountain and floodplain regions. Herded and transhumant domestic livestock, which was probably the most important dispersal vector in the Central European man-made landscape for centuries, dramatically decreased or vanished locally completely.

Whereas in the traditional man-made landscape all habitats were more or less connected due to alternating management practices or grazing today most habitats are isolated. The consideration of dispersal processes or vectors should therefore be one basic element in the restoration of habitats. If there is no possibility to restore traditional dispersal processes these processes have to be simulated or replaced by other ones which may also include the artificial reintroduction of species.

Introduction

Until now species-richness in plant communities is regarded as a function of abiotic factors characteristic for a habitat or ecosystem, such as hydrological conditions, nutrients and light, as well as different biotic factors, such as mycorrhiza (ELLENBERG 1996; OZINGA et al. 1997). Further hypotheses were summarized by ZOBEL (1992). Most of these hypotheses relating to species coexistence assume that all species have the same mobility or even that each species can reach all suitable habitats. Although it is obvious that this is unlikely it was supposed by most vegetation ecologists* and even in textbooks on dispersal ecology (RIDLEY 1930; MÜLLER-SCHNEIDER 1977; VAN DER PIJL 1982).

However, only few years ago the species pool-concept claims that dispersal is limiting species-richness in plant communities (PÄRTEL et al. 1996; ZOBEL 1997). Recently BONN & POSCHLOD (1998a,b) and POSCHLOD & BONN (1998) reviewed the processes which could be responsible for the dispersability of plant species taking into account that nearly all plant communities in Central Europe are man-made or at least modified by man. In any case, it is clear that land use practices combined with dispersal vectors such as livestock are more important for the dispersal of plant species in comparison to their own dispersability as pronounced in the above mentioned textbooks.

This paper analyses the processes existing in the natural (in Central Europe after the last ice age), the traditional and the actual man-made landscape which are important for the dispersal of plant species (see also BONN & POSCHLOD 1998a,b; POSCHLOD & BONN 1998). The comparison of the changing number and type of dispersal-relevant processes during the evolution of the Central European man-made landscape since the last glaciation should allow a first critical assessment of the importance of these processes for the species richness of plant communities especially in the context of restoration management.

Dispersal in the postglacial vegetation

During the postglacial period before human settlement, there was a massive change in the composition of vegetation. However, although the change of vegetation is quite well known through palynological research (LANG 1994), there are only a few ideas on the dispersal and spread of plant species during that time. Consequently, KOLLMANN (1992) calls the middle European „Grundfolge“ (postglacial vegetation development after FIRBAS 1949) the most speculative application of the knowledge on dispersal ecology of plants.

Important dispersal vectors in the natural landscape were wind, water and animals. Whereas in an open tundra landscape wind could have been an important factor for plant dispersal this was

* The classification of plant communities (plant sociology) is based on the occurrence of distinct species in their habitats (character species) although there are no ideas which dispersal processes and vectors are responsible for its distribution (DIERSCHKE 1994). In contrast, GLEASON already mentioned n 1926 that species composition of vegetation is the result of migration ability and environmental sorting (see also SALISBURY 1964).

not the case in a wooded landscape, where wind probably only caused patterns of plant distribution on a small scale. Water was likely a more important dispersal vector during that time. Whereas trees and shrubs are regarded as hydrochorous (RIDLEY 1930; DELCOURT & DELCOURT 1991; LANG 1994), water is usually not taken into account as a relevant dispersal vector for herbaceous species. However, FUCHS (in POSCHLOD et al. 1997) found germinable diaspores of 63 species in drifted material and in the sediment of the Upper Loire river (France), most of them species from open habitats like disturbed places and grasslands (Table 2.1). In a study of the seasonal variation of drifted diaspores in little rivers more than 80,000 diaspores per day were caught in a 15x24 cm² area represented by the size of a drift net (Table 2.2; TROTTMANN & POSCHLOD, unpublished data). In both studies, most of the species were not known to be dispersed by water before. Only four of 63 species in the first study and 12 of 43 species in the second study had been regarded as nautochorous so far (MÜLLER-SCHNEIDER 1986).

Table 2.1 Number of species with germinable diaspores in drifted material and in the sediment (sand, gravel) in a region of the Upper Loire in the south of Le Puy (France, after POSCHLOD et al. 1997).

Vegetation types	Only in the drifted material	In the drifted material and the sediment	Only in the sediment	Total
Freshwater and peatland vegetation	1	1	2	4
Vegetation of disturbed places	4	13	3	20
- Artemisietea	2	6	1	9
Alpine vegetation	-	-	2	2
Grassland	3	8	3	14
- Sedo-Scleranthetea	-	5	2	7
- Festuco-Brometea	-	-	1	1
- Molino-Scleranthetea	3	2	-	5
Fringe and shrubland	-	1	2	3
Woodland	1	3	-	4
Species from other vegetation types	2	5	9	16
Total number of determinable species	**11**	**31**	**21**	**63**

Animals are assumed to have been the most important dispersal vectors in the postglacial time, especially with respect to long distances. According to MÜLLER-SCHNEIDER (1949), SAUER (1986), JOHNSON & ADKISSON (1988) and JOHNSON & WEBB (1989) most trees and shrubs may have

been spread by birds. DARLEY-HILL & JOHNSON (1981) and MATTES (1982), for example, reported that 54% and up to 60% respectively of the whole diaspore production of oaks and *Pinus cembra* can be dispersed by different species of jay. Large herbivores are regarded even as more effective with respect to the number of species dispersed (JANZEN 1981; MALO & SUÁREZ 1995), which concerns predominantly non-woody plant species (PAKEMAN 2001). In this context HERRERA (1989) stated that not only herbivores but also carnivorous animals acted as very important dispersal vectors during postglacial time. This hypothesis is supported by ROGERS & APPLEGATE (1983) and WILLSON (1993a), who found thousands of germinable diaspores in only one dung deposit of black beers. Most existing studies concerning dispersal by animals concentrated on endozoochorous dispersal. However, MROTZEK et al. (1999) and HEINKEN & RAUDNITSCHKA (2002) demonstrated for wild boar, that many species of open habitats are also transported epizoochorously. Furthermore, FISCHER et al. (1995, 1996) and STENDER et al. (1997) showed, though only for livestock, that epizoochorous dispersal on fleece or fur and by hoofs can be much more important than endozoochorous dispersal with respect to the number of diaspores and species dispersed.

Table 2.2 Number of diaspores drifted by open water per day and 15x24 cm² area (size of the water body which was caught by a drift net) in little rivers of the plainlands around Munique and Augsburg.

Study period: June 1995 to February 1996; data from TROTTMANN & POSCHLOD, unpublished.

time of sampling / site	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Febr.
Viertelsgraben (upper reaches)	1,464	504	864	144	312	144	240	24
Viertelsgraben (central reaches)	2,784	10,368	1,272	1,152	1,080	960	648	4,896
Pfullinger Graben	1,968	3,648	432	1,008	1,152	672	288	384
Vorflutgraben, Nord	-	6,624	744	96	144	48	576	-
Friedberger Aach (central reaches)	24,624	82,512	50,832	21,744	22,032	32,472	19,008	20,160
Moosach (central reaches)	26,064	55,008	18,000	11,664	12,672	16,272	15,120	27,792

Diaspores dispersed by animals can be transported over a long time period (SHMIDA & ELLNER 1983; SIMAO NETO et al. 1987; RUSSI et al. 1992; FISCHER et al. 1996). During that time animals can cover long distances depending on their migration behaviour (FISCHER et al. 1996; KIVINIEMI 1996). Since we know that the number of species of large herbivores and carnivores

decreased continuously from the postglacial time until the 17th century due mainly to the impact of man (BEUTLER 1996), we have probably lost many important dispersal vectors for plants. In this context it is important to state that in addition to diaspore dispersal these animal species created the germination niches and the habitats for most of the dispersed plant species by disturbing the turf through trampling.

Aspects of diaspore dispersal in the man-made landscape

With the beginning of the Neolithic age and the settlement of man a continuous period of rapid changes of landscape and vegetation began. Along with the settlement of man natural processes decreased, whereas the separation of functions and processes as well as the fragmentation of habitats and active land use management increased. DI CASTRI (1989) summarized the most important processes which were the driving forces for the spread of plants and animals since the Neolithic Revolution up to the 15th century, from the 15th century until the 18th century, and since the 19th century with the introduction of intensive agricultural practices, trade and traffic. One of his conclusions was that since the beginning of human settlement the globalisation and acceleration of dispersal processes increased. Compared to the vegetation changes during the early postglacial time the speed of floristic change caused by anthropogenous dynamics is tremendously higher (Fig. 2.1).

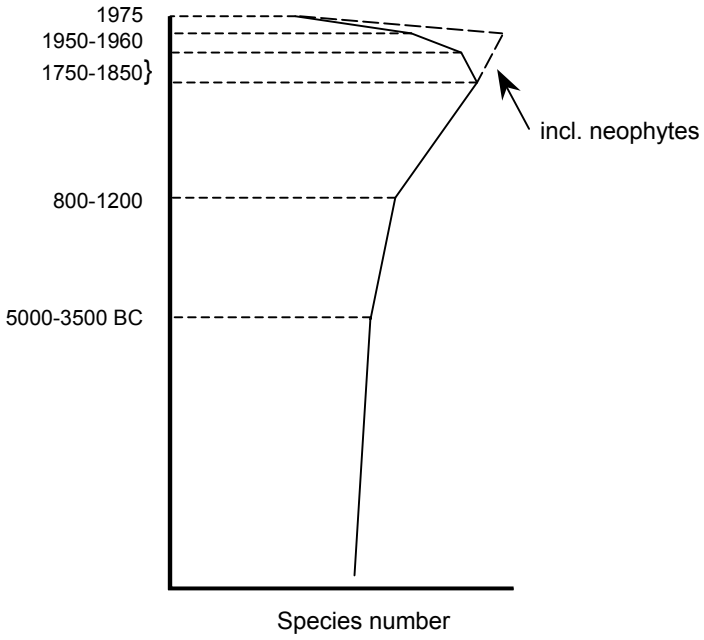

Fig. 2.1 The change of the number of plant species since the last glaciation.
Broken line: neophytes; from SUKOPP & TREPL (1987) after FUKAREK (1979).

After the steady increase of species diversity since the beginning of human settlement the climax was reached in the middle of the 19th century. Whereas the decline of indigenous species could first be compensated by introduced neophytes, an absolute decline of plant species diversity can be observed since the middle of the 20th century (Fig. 2.1; FUKAREK 1979; SUKOPP & TREPL 1987). Again, the rapid changes of dispersal processes in the man-made landscape have to be regarded as one important reason for this tremendous decline of species diversity starting in the middle of the 19th century.

Dispersal by agricultural practices

Agriculture, which formerly also included the use of forests, was the most important form of land use over millennia and therefore had a formative influence on most parts of the Central European landscape. Because of this dominant influence of agricultural land use, this paper considers in the following the change of different agricultural practices affecting the dispersability of plants.

Dispersal by sowing seed

Many weeds which were formerly very common were spread by contaminated seed (speirochory). Although simple methods of seed cleaning already existed in the Neolithic age (KNÖRZER 1971), seed cleaning remained very ineffective until the end of the 19th century. Over millennia, crop seed therefore contained high amounts of weed diaspores (WITTMACK 1888; SCHNEIDER et al. 1994). A rye-finding from the 17th century, for example, consisted of 12% *Bromus secalinus*- and 2% *Agrostemma githago*-diaspores (WITTMACK 1888; HOPF 1972). STEBLER (1878) still wrote at the end of the 19th century about *Bromus secalinus*: “In cereal fields it is an extremely harmful weed and occurs in wet years together with the bearded darnel in such huge numbers that the legend originated of cereal being converted into brome“. One method of seed cleaning, existing since Neolithic age (KNÖRZER 1971) until the present, is based on differences in the terminal velocity of diaspores, which is a result of diaspore size, weight and morphology (TACKENBERG 2001). Because only diaspores remaining in the crop were sown together with the seed, weeds with diaspore characteristics similar to those of the crop (large, heavy diaspores), which could not be separated by traditional methods of seed cleaning, were selected (BARRETT 1983).

Not only the seed of annual arable crops was highly contaminated, but also clover and grass seed. At the end of the 19th century, for example, about 90% of all clover seed from Austria and Silesia were contaminated with diaspores of *Cuscuta epithymum* ssp. *trifolii* (STEBLER & SCHRÖTER 1902), a species, which is endangered today. Grass seed studied by KORSMO (1930) was contaminated with up to 424,000 diaspores of undesired species per kilogram seed. SALISBURY (1953) estimated the number of diaspores sown by contaminated clover and grass seeds in the first decade of the 20th century in Great Britain between two and six billions per year!

Table 2.3 Rare or endangered weed species dispersed by seed (SCHNEIDER et al. 1994).
1): WEHSARG (1918), dispersal with seed: - never, + = rarely ++ = yes; +++ = frequently; **2)**: KORNAS (1972, 1988), dispersal with seed: + = occasional; ++ = facultative; +++ = obligatory; **3)** dispersal with seed (x) according to other authors (after SCHNEIDER et al. 1994); (s): only in seed of summer grain, (f) only in flax seed (WEHSARG 1918, KORNAS 1988); persistence in the diaspore bank (SCHNEIDER et al. 1994, THOMPSON et al 1997): ts = transient, sps = short-term persistent, lps = long-term persistent; status in the German Red Data book (KORNECK et al. 1996): 0 = extinct; 1 = threatened of being extinct, 2 = heavily endangered, 3 = endangered, - = not endangered.

species	frequency / occurrence in seed			persistence in the diaspore bank	status in the German Red Data book
	1)	2)	3)		
Adonis aestivalis	+++		x	sps?	3
Adonis flammea	+++			?	1
Agrostemma githago	+++	+++	x	ts	1
Ajuga chamaepitys			x	?	3
Bromus arvensis			x	ts	3
Bromus grossus			x	?	1
Bromus secalinus	+++	++	x	ts	-
Bupleurum rotundifolium	-		x	?	1
Camelina alyssum (f)		+++		ts	0
Caucalis latifolia	++			?	1
Caucalis platycarpos	++			ts/sps	3
Centaurea cyanus	+	++	x	sps	-
Chrysanthemum segetum (s)	+		x	lps	-
Conringia orientalis	++		x	sps	2
Consolida regalis	++		x	lps?	3
Cuscuta eplinum (f)		+++		lps	0
Fagopyrum tataricum		+++	x	?	?
Galeopsis ladanum (s)	++		x	?	-
Galeopsis segetum (s)	++			?	-
Galium parisiense			x	ts	0
Galium spurium ssp. spurium (f)		+++		?	0
Galium tricornutum	+++		x	ts	3
Lathyrus aphaca	+++			?	3
Legousia speculum-veneris (s)	+		x	ts	3
Lithospermum arvense ssp. *arvense*	++		x	ts/sps	-
Lolium remotum (f)		+++		?	0
Lolium temulentum (s)	+++	+++	x	ts	0
Melampyrum arvense	++			sps	-
Neslia paniculata ssp. *paniculata* (s)	++			lps	3
Nigella arvensis	+		x	?	2

Table 2.3 continued Rare or endangered weed species dispersed by seed (SCHNEIDER et al. 1994).

species	frequency / occurrence in seed			persistence in the diaspore bank	status in the German Red Data book
	1)	2)	3)		
Orlaya grandiflora	++			?	1
Ranunculus arvensis				ts	3
Rhinanthus alectorolophus ssp. *buccalis*	+++	+++	x	sps?	-
Rhinanthus serotinus ssp. *apterus*		+++	x	sps?	-
Scandix pecten-veneris (s)	+			ts/sps	2
Sherardia arvensis	+	+		ts	-
Silene gallica		+		sps	-
Silene linicola (f)		+++		?	0
Stachys annua (s)	+			?	3
Stachys arvensis (s)	+			lps	3
Torilis arvensis	++			sps	-
Vaccaria hispanica ssp. *hispanica* (s)	+++		x	sps/lps	1

With the beginning of the 20th century seed cleaning was markedly improved by new cleaning methods, which SALISBURY (1964) called “one of the milestones in the history of weeds”. According to the rules of the seed prescription cereal seed must have a purity of at least 98% or 99% at certificated and base seed respectively (FUCHS et al. 1979; KUHNHARDT 1986). The massive improvement of seed cleaning led from “the ecological specialisation to the extinction” (KORNAS 1988) or at least endangerment of many weed species (Table 2.3) which are specialized on dispersal via seed. The selection towards the production of few, but heavy and large diaspores, which was caused by the traditional methods of seed cleaning (see above), is disadvantageous with regard to other ways of long-distance dispersal (KORNAS 1988; SCHNEIDER et al. 1994). A persistent diaspore bank could be an alternative strategy to survive in an arable weed community. Most of the typical speirochorous species, however, lack this regeneration strategy (Table 2.3). For many species, which are rare or endangered today (Table 2.3) dispersal via seed was therefore a very important if not the only way of dispersal.

Species, which are regarded as “wild plants” or “weeds” today were not only dispersed unintentionally by contaminated seed but also deliberately introduced by man. In grasslands, for instance, additional sowing was in former times done by hayseeds which were collected in the barn, fallen out from the stored hay (STEBLER 1878). Before the availability of commercial grassland seed this was the only possibility to establish new grasslands (SCHRÖDER-LEMBKE 1983). The consequence of the simple collection of diaspores fallen out of the stored hay was that species of a big variety of habitats and different populations, consisting of “weeds and crops, of useful, noxious and worthless elements” (STEBLER 1878) were dispersed by man. Although in the middle

of the 19th century hay seed was no longer recommended (HÄFENER 1847), new grasslands were still established by this method until the beginning of the 20th century, e.g. species-rich mountain meadows (HARD 1964) and calcareous grasslands on abandoned vineyards (SCHUMACHER et al. 1995). According to investigations of KAUTER (2002) even the weed diaspores obtained during the cleaning of grain seed were occasionally sown to establish temporary pastures.

Furthermore, several "wild" species were cultivated for a certain period and therefore dispersed intentionally by man. Species-rich litter meadows, for example, today an endangered habitat with a lot of rare species (KORNECK & SUKOPP 1988), were artificially established not only by sowing but also by planting (STEBLER 1898). This was done especially in those regions where arable land to gain straw as litter was missing, such as in the foothills of the Alps. *Molinia caerulea*, the dominant species of the litter meadows in this region, which seeds were collected and sown, almost became a cultivated plant (STEBLER 1898; KONOLD & HACKEL 1990). Other species, which were temporary cultivated, are *Anthyllis vulneraria, Onobrychis viciifolia* or *Bromus secalinus* (STEBLER & SCHRÖTER 1895, 1902; SALISBURY 1964; BURRICHTER et al. 1993)

Dispersal by different fertilizers

The chronic lack of fertilizer as well as the continuous defile of litter were characteristic for the historical agriculture, leading to the use a big variety of substitutes which were spread on the arable land and at least part of the grassland (HÄFENER 1847; KAUTER 2002). This use of substitutes also guaranteed the dispersal of species between different habitats of the historical man-made landscape (Fig. 2.2, Fig. 2.3).

Manure, which was the most widespread fertilizer in historical times, contained a lot of diaspores depending on which materials were put on the manure heap (KORSMO 1930). Typically, it consisted of a big variety of materials used as litter, such as sods from heathlands, forest or peat litter, chaff, hay from litter meadows etc., as well as different wastes, fodder remains and the excrements of livestock (Fig. 2.2). Already the dung of livestock necessary to fertilize 1 ha of an arable land contained several hundred thousands of diaspores (KORSMO 1930, Table 2.4). Further fertilizers, spread without a previous fermentation on the field, were gained from various habitats and wastes, such as sods from heathlands and forests, peat, freshwater mud as well as compost, rubbish etc. (Fig. 2.2). Each of these fertilizers was equipped with a certain amount of diaspores (Table 2.4), which were all brought out on the fields.

Today, a direct transfer of fertilizers from other habitats, such as heathlands or forests, to the arable land does no more exist. Mineral fertilizers or animal slurry, containing no or only few diaspores, are the dominant fertilizers applied to fields (Fig. 2.2). The most obvious cause for the low diaspore content in slurry as well as in manure is that grass for fodder-production (hay, silage) is today mown earlier and more often. Consequently most plant species cannot reproduce until mowing. Furthermore, longevity of diaspores in animal slurry is often lower than in manure due to storage period, storage temperature and the toxicity of ammonia (DORPH-PETERSEN & HOLMGAARD

1928; RIEDER 1966a,b, 1985; CHYTIL 1986; KELLERER & ALBRECHT 1996). KELLERER & ALBRECHT (1996) found an average content of 3.4 diaspores/l slurry. With 30,000 l slurry, the amount to fertilize 1 ha, 10,200 diaspores are brought out on the arable land, thus far less than with the fertilizers used in the historical man-made landscape. Additionally, the toxicity of ammonia leads to a strong selection of species with hard seed coats, which are less sensitive to ammonia (RIEDER 1966a). This means that we do not only have a lower input of diaspores by spreading slurry or mineral fertilizer instead of manure, but also an input of viable diaspores different in composition and dominance.

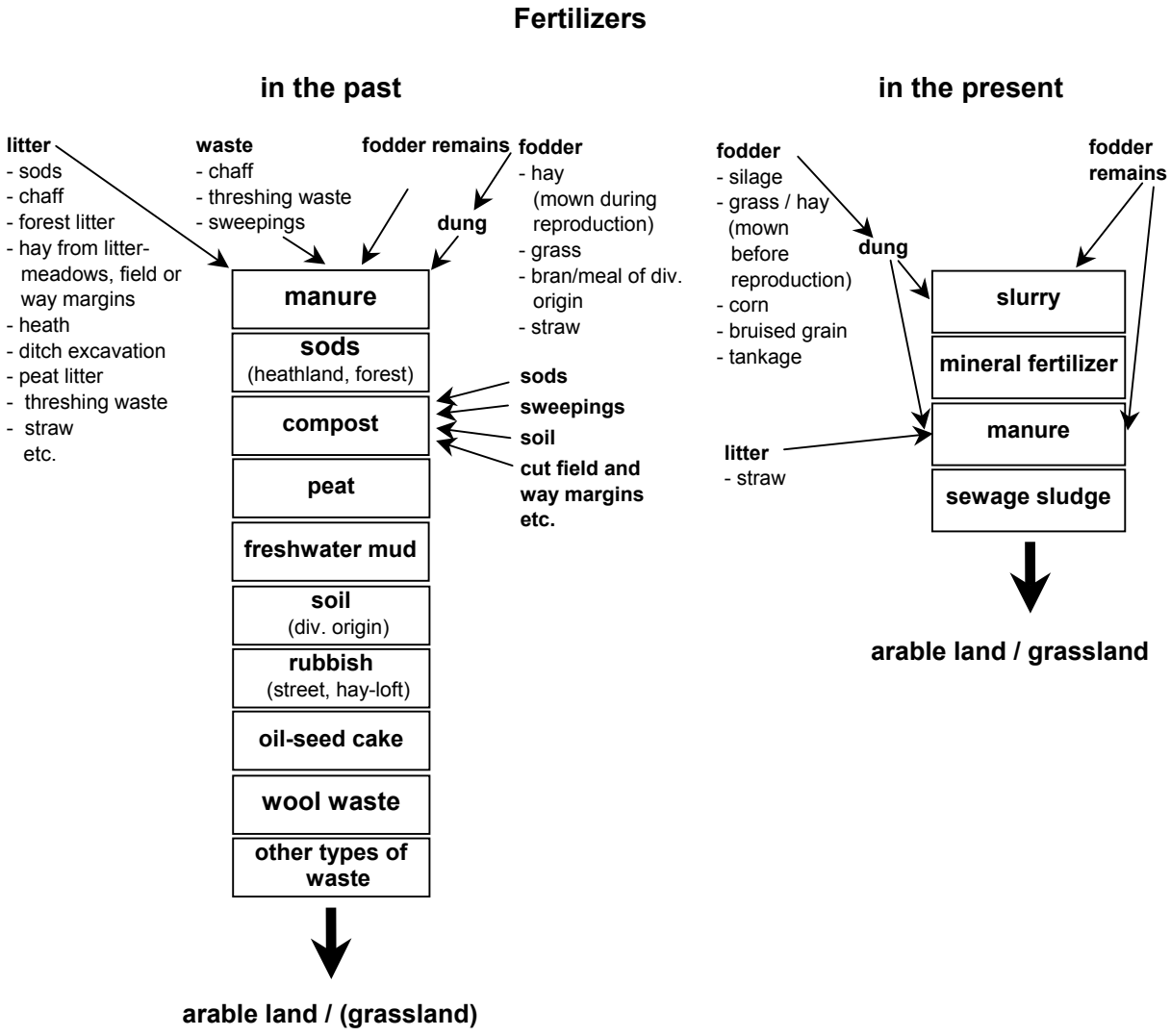

Fig. 2.2 Fertilizers applied to arable land and grassland in the past and in the present.

Table 2.4 Diaspore contents of different materials used as fodder, litter or fertilizer for arable land or grassland in the historical man-made landscape (data from KORSMO 1930, CHYTIL 1986, POSCHLOD et al. 1996b).

*: amount which was used to fertilize 1 ha arable land (according to KORSMO 1930).

Materials used as fodder, litter or manure	Diaspore content	Number of species
Threshing waste	16,500 - 1,734,500/kg	14-27
Chaff	4,500 - 170,000/kg	?
Hay-loft sweepings	182,500/kg	13
Straw fodder / litter	no given number	10-17
Bran / meal	80 - 6,800/kg	?
Scouring waste from mills	287,800/kg	22
Horse dung (storage < 0,5 years)	326,440 - 958,960/60t*	?
Cattle dung (storage < 0,5 years)	58,960 - 488,230/60t*	?
Pig dung (storage < 0,5 years)	326,440 - 511,490/60t*	?
Sheep dung (storage < 0,5 years)	825,000/60t*	?
Hen dung (period of storage unknown)	1,042,039/60t*	?
Compost (dung and soil from field margins, roadsides etc.)	19,000,000/40t*	?
Pond mud	>6,000/l	up to 42

A special form of fertilization of meadows was artificial flooding. This flooding of meadows in flood plains and even in mountain regions after snow melting or heavy rainfall events as well as for irrigation in dry periods was very common in Central Europe until the 20th century (KLAPP 1971). Because studies concerning diaspore dispersal by artificial flooding do virtually not exist, there are only some ideas about the effects of such flooding events on species richness of these meadows by dispersing diaspores. More than 30 species could be found dispersed by water during an experiment after artificially flooding meadows in the Eder river valley near Marburg (Germany) in June using the old irrigation ditch system (POSCHLOD, unpublished data). KELLY & BRUNS (1975) identified in ditches irrigating arable land in North America 84 to 136 species in one year. The diaspore quantities extended from 268 to 875 diaspores/100 m³ irrigation water. In a comparable study, WILSON (1980) documented up to 964 diaspores in 100 m³ water. SCHWABE-BRAUN (1980) and SCHÜLE & SCHWINEKÖPER (1988) showed that *Genista tinctoria*, *Hypericum perforatum* and *Tussilago farfara* established in mountain meadows after dispersal by the irrigation water.

In 1941/1942 more than 20% of the agricultural land was still flooded artificially in some regions, e.g. in the Black Forest and Southern Westphalia (KLAPP 1971). However, after the World War II this traditional land use form became almost totally lost. Today, the abandonment of artificial flooding is regarded as one reason for the increasing losses of grassland species (KORNECK & SUKOPP 1988).

Dispersal by harvest methods

The way of dispersal of weeds growing on a field (e.g. by seed) was also largely determined by the methods of crop harvesting. In the Neolithic age cereal crop was picked by hand or cut by a harvest knife (JANKUHN 1969). By this harvest method only weed species with a release height similar to the crop plant were harvested and spread again by contaminated seed (WILLERDING 1986, see above). This lead to a rapid areal expansion of tall weeds such as *Agrostemma githago* (KNÖRZER 1971).

Not before the iron age the crop was harvested close to the soil surface by a sickle. Then also low growing or prostrate weeds got the chance of being spread over long-distances by contaminated seed (KNÖRZER 1971; WILLERDING 1986). The method of harvesting did not change for a long time. The crop was harvested by mowing during yellow ripeness, corn sheaves were bound and dried on the field and then cart off. Threshing, separation from straw, cereal seed and chaff were done at the farm. Part of the weed diaspores remained on the field (KORSMO 1930; PETZOLDT 1957, 1959), where they were spread by binding and setting the sheaves. The weed diaspores which remained in the corn sheaves were transported to the farm and threshed together with the crop. Partially the weed diaspores remained in the threshing and were spread again on the field by sowing the seed contaminated with weed diaspores (Table 2.3). The rest of the weed diaspores got into the chaff or other threshing waste which were used as fodder, litter or put on the manure heap (Fig. 2.2). The chaff could contain up to 170,900 weed diaspores per kilogram (Table 2.4), the other threshing waste more than 1,734,500 weed diaspores per kilogram (KORSMO 1930). These remains from threshing were either fed, used as litter or directly thrown on the manure heap, from where they were spread again on the field, if they were still alive. The spread of chaff on the arable land, for example, was the cause of the rapid spread of *Bromus tectorum* in North America (MACK 1981). Altogether, all weed diaspores produced on the arable land remained either on the field or were dispersed again at the different fields of a farm after threshing if they were able to survive e.g. storage in manure.

Since the end of the 1960s most cereals were harvested by combines, by which the drying of corn-sheaves on the field became unnecessary. Therefore, cereal was harvested two to three weeks later, during full ripeness (BACHTHALER 1968). Chaff and straw became already separated on the field. Consequently most of the weed diaspores remained on the same field. Due to the later harvest also weed species had more time for diaspore maturation and diaspore shedding. Consequently, those species became dominant which shed their diaspores until the later date of harvesting, such as *Avenua fatua*, *Apera spica-venti*, *Alopecurus myosuroides* (DOLLINGER 1988; ALBRECHT 1989) or *Atriplex patula*, *Galeopsis tetrahit*, *Lapsana communis* and *Vicia angustifolia* (PETZOLDT 1957, 1959). Several of those species became for the first time problematic weeds since that time. The massive increase of *Avena fatua*, for example, which was once nearly extinct, is attributed to the later harvest date of cereals (ELLENBERG 1996). In contrast, typical speirochorous species, such as *Agrostemma githago, Bromus secalinus* or *Rhinanthus alectorolophus* ssp. *buccalis* keep their diaspores at the infructescence even after seed or fruit maturation.

They were therefore harvested together with the cereals and had due to improved methods of seed cleaning no chance to return to the arable land.

Table 2.5 Behaviour of different weed diaspores in the air blast of a wind channel in relation to their frequency in chaff (PETZOLDT 1957).

species	trajectory similar to chaff	diaspore content of chaff	species	trajectory similar to chaff	diaspore content of chaff
Melandrium noctiflorum	0%	missing/low	*Echinochloa crus-galli*	67%	very high
Caucalis platycarpos	0%	missing/low	*Poa trivialis*	81%	very high
Conringia orientalis	0%	missing/low	*Galinsoga parviflora*	83%	very high
Galium tricornutum	0%	missing/low	*Rumex crispus*	83% (with perigon)	very high
Lithospermum arvense	0%	missing/low	*Poa annua*	84%	very high
Melampyrum arvense	0%	missing/low	*Rhinanthus minor*	84%	very high
Avena strigosa	0%	missing/low	*Apera spica-venti*	88%	very high
Euphorbia helioscopia	0%	missing/low	*Sonchus* spec.	88% without, 100% with pappus	high
Geranium dissectum	0%	missing/low	*Holcus mollis*	90%	very high
Adonis aestivalis	1%	low	*Matricaria perforata*	94%	very high
Bromus secalinus	5%	low	*Matricaria recutita*	100%	very high

Another effect is attributed to the separation of grain from chaff in the combine harvester. This separation is done by air blast, resulting in a blowing out of the lightest weed diaspores together with the removed chaff (e.g. *Apera spica-venti*, *Poa annua*, *Matricaria recutita*, *Papaver rhoeas*, *Rumex crispus*, *Sonchus arvensis*, Table 2.5). In contrast, heavy and relatively big diaspores with a high terminal velocity, such as the typical speirochorous species, were carried away in the grain tank of the combine (PETZOLDT 1957; AAMISEPP et al. 1967; FOGELFORS 1982, WACKER 1989). About 70% to 75% of all weed diaspores which are still kept at the infructescence during combine harvest remain in the grain tank, the rest is blown out at the field together with the chaff, waste or straw (PETZOLD 1957). This example shows that the change of land use practices also resulted in a selection of plant species due to their size and weight of diaspores, those with big and heavy diaspores over those with small, light diaspores with a low terminal velocity (AAMISEPP et al. 1967; DOLLINGER 1988).

Finally, the different harvest methods influenced the dispersal patterns of weed species on the arable land, which may have an impact on population growth (e.g. BALLARÉ et al. 1987; MCCANNY & CAVERS 1988). Due to the dispersal of diaspores over long distances, harvesting by combine lead from a clumped to a more homogenous distribution of weed diaspores, which was

demonstrated for *Datura ferox* (BALLARÉ et al. 1987), *Bromus interruptus* and *B. sterilis* (HOWARD et al. 1991; GHERSA et al. 1993).

Another example of dispersal by machines was given by STRYKSTRA et al. (1996), who showed that hay-making machinery is an important dispersal vector whereas mowing by scythe had no seed dispersal effect.

Dispersal by livestock

After the loss of numerous wild ungulates, domestic livestock has according to RIDLEY (1930) taken over the function as a dispersal vector for plant species. In the historical man-made landscape these grazing "domestic dispersal vectors" were more or less ubiquitous. Because feed usually was rare, a big variety of habitats had to be used at least temporary as pasturages for livestock, e.g. the arable land, fallows, heath- and peatlands, forests a.o. (e.g. Fig. 2.3). The dispersal potential of these mobile vectors has been demonstrated by numerous studies (s. overview in BONN & POSCHLOD 1998a). Based on the results of these studies an evaluation of zoochorous dispersal potential of the character species of anthropogenous heaths and grasslands (according to ELLENBERG 1996) shows that at least 90% of the character species of the Molinio-Arrhenatheretea and at least 70% of the species of the Nardo-Callunetea have been proved of being dispersed by animals in previous studies (BONN & POSCHLOD 1998a).

Table 2.6 Number of species from grasslands and other habitats (total) dispersed by Galloway cattle and sheep in northwest German lowland dry and wet grasslands and southwest German dry calcareous grasslands respectively (from STENDER et al. 1997; FISCHER et al. 1995, 1996).
[1]: the total amount of transported seeds of sheep is higher because they grazed also on arable land, roadsides a.o., whereas the Galloway cattle only grazed on grassland.

	Number of species dispersed by			
	Galloway cattle		sheep	
	from grasslands	total	from grasslands	total[1]
Fleece / fur	29	29	44	86
Hoofs	41	41	36	47
Dung	57	57	20	28
Total	69	69	57	109
proportion of the local grassland species pool which produced diaspores	50%		52%	

From a local species pool of 118 plant species from calcareous grasslands on the Swabian mountains in Southwest Germany diaspores of 57 species (52% of the local species pool producing diaspores; Table 2.6) were dispersed by sheep either epi- or endozoochorously (FISCHER et al. 1995, 1996). In total, more than 8,500 diaspores were found in the fleece, 382 in the hooves and 273 in the dung of sheep. However, except for the collected dung, this study was done only on one tamed sheep of a flock of about 350 sheep and most species were dispersed in only small numbers. Therefore, it is clear that a far higher number of species was dispersed, probably more or less all grassland species. FISCHER et al. (1995) calculated that more than 3,000,000 diaspores were dispersed by a flock of 350 sheep during only one vegetation period.

Diaspores attached in the fleece can be transported for about hundred days (FISCHER et al. 1995). The distances covered by domestic livestock during that time could amount up to hundreds of kilometres in former times. Transhumant herding was formerly widespread in many European countries and herding trails to the livestock-markets went through whole Central Europe (HORNBERGER 1959; WIESE 1966).

Taking into account these facts, it becomes obvious that dispersal of species, at least of those from grazed areas, was not a limiting factor for the survival of plant populations during that time. This assumption is also supported by studies concerning the vegetation development of abandoned arable land after reintroduction of sheep grazing which caused the invasion of a lot of species which were present neither in the actual vegetation nor in the seed bank (GIBSON et al. 1987). Until now, however, no study does exist which is able to prove the extinction of local populations or even species due to the lack of dispersal by livestock. There are only assumptions such as those from KRAUSS (1977) who attributes the decrease of *Chenopodium bonus-henricus*-populations to the ending of the former migrating sheep and goats. Similar causes are supposed by MATTHIES (1984) with respect to the actual rarity of *Melampyrum cristatum* in calcareous grasslands. JANSSEN (1992) concludes from the species sets of isolated calcareous grasslands in the Harz Mountains (Germany) that formerly grazing flocks of sheep were responsible for a diaspore exchange between single calcareous grasslands, leading to a similar species composition of these habitats.

Management of livestock by transhumant or local herding, however, vanished almost totally throughout Central Europe during the last decades and was replaced by stable or paddock management (ERDMANN 1983; BEINLICH & PLACHTER 1995). Only in some places local herding is still occurring. Additionally, the number of livestock, especially sheep, decreased tremendously at the beginning of the 20th century (Germany - 1870: 25 millions of sheep, 1907: 5 millions of sheep; LAHRKAMP 1928) due to cheap imports of wool, fleece and meat, even from overseas.

The effects of the abandonment of local or transhumant shepherding on plant populations are described by JANSSEN (1992): after the loss of the "moving ecological infrastructure" (POSCHLOD et al. 1996a) completely isolated plant populations remained. In consequence, local extinctions of populations first lead to an increasing differentiation of single calcareous grasslands, but long-term to an increasing loss of species because of a missing immigration of plant species via long-distance dispersal.

Dispersal processes between habitats

The traditional man-made landscape was characterized by a big variety of alternating managements, which on the one hand made the landscape more "passable" (s. HARD 1964) and on the other hand facilitated dispersal processes between habitats, because most habitats were connected by dispersal processes (Fig. 2.3).

Arable fields, for example, were not used continuously, but as alternating arable land-fallow- or arable land-pasture-systems. The fields were usually used only for some years and then abandoned or grazed by livestock (ABEL 1978; POTT 1988). Depending on soil fertility, type of management and distance to the village, fallow or grazing period could last up to 40 years. These temporary interruptions connected the arable land to other habitats.

A shifting management also occurred in forest habitats such as coppices in southern Westphalia. After coppicing sites were burned and used as arable land to grow cereal and buckwheat for one or two years. During the fallow period *Sarothamnus scoparius* was spreading and used as fodder for sheep and litter for the stables. When the tree canopy began to dominate again the coppice forest was grazed for some years before it was cut again (POTT 1985). Shifting cultivations also existed in heath- and peatlands. In these farming systems the heath or peat was burnt for fertilization and the area was cultivated for one or several years. After crop cultivation, the fields were grazed for several years and afterwards completely abandoned until the next rotation (e.g. ABEL 1978; SELTER 1995). Each of these land use forms was connected with the dispersal of diaspores, e.g. by grazing livestock, contaminated seed or fertilizer (s. BONN & POSCHLOD 1998a).

Artificial flooding connected not only meadows to each other (KLEINSCHMIDT & ROSENTHAL 1995) but more or less all habitats which were adjacent to the ditches. Sometimes artificial ponds were established in the vicinity of farms in which slurry and liquid manure were poured into to irrigate or fertilize the meadows (ENDRISS 1952). Furthermore, the mud of the ditches as well as of ponds was used for fertilization (Fig. 2.3). The forests were not only used for the production of timber, but also for litter production and pasture.

Taking into account that livestock was extremely widespread in the traditional man-made landscape, it probably was the most important local and regional dispersal vector. BURRICHTER et al. (1993) distinguish various forms of local herding, practiced until modern times:

- grazing in woods and resulting pastoral woodlands including dry grasslands, heath- and pasture-lands;
- wood-pasture in the context of forest-shifting agriculture;
- grazing on several year-old fallows in the context of arable land-pasture farming as well as in the temporary fire-cultures in peat- and moorlands;
- grazing on fallows in the context of the three-field rotation;
- grazing on stubble fields after the harvest;
- temporary winter-grazing on arable land sown with winter crops in order to promote tillering.

Livestock therefore connected not only arable land and grasslands, but also heathlands, peatlands and forests. Of 86 plant species indicating forest grazing in the Bavarian Alps (according to STORCH 1983; LISS 1988 and RÖSCH 1990) 64 species are dispersed epizoochorously and 40 species endozoochorously by livestock (BONN & POSCHLOD 1998a).

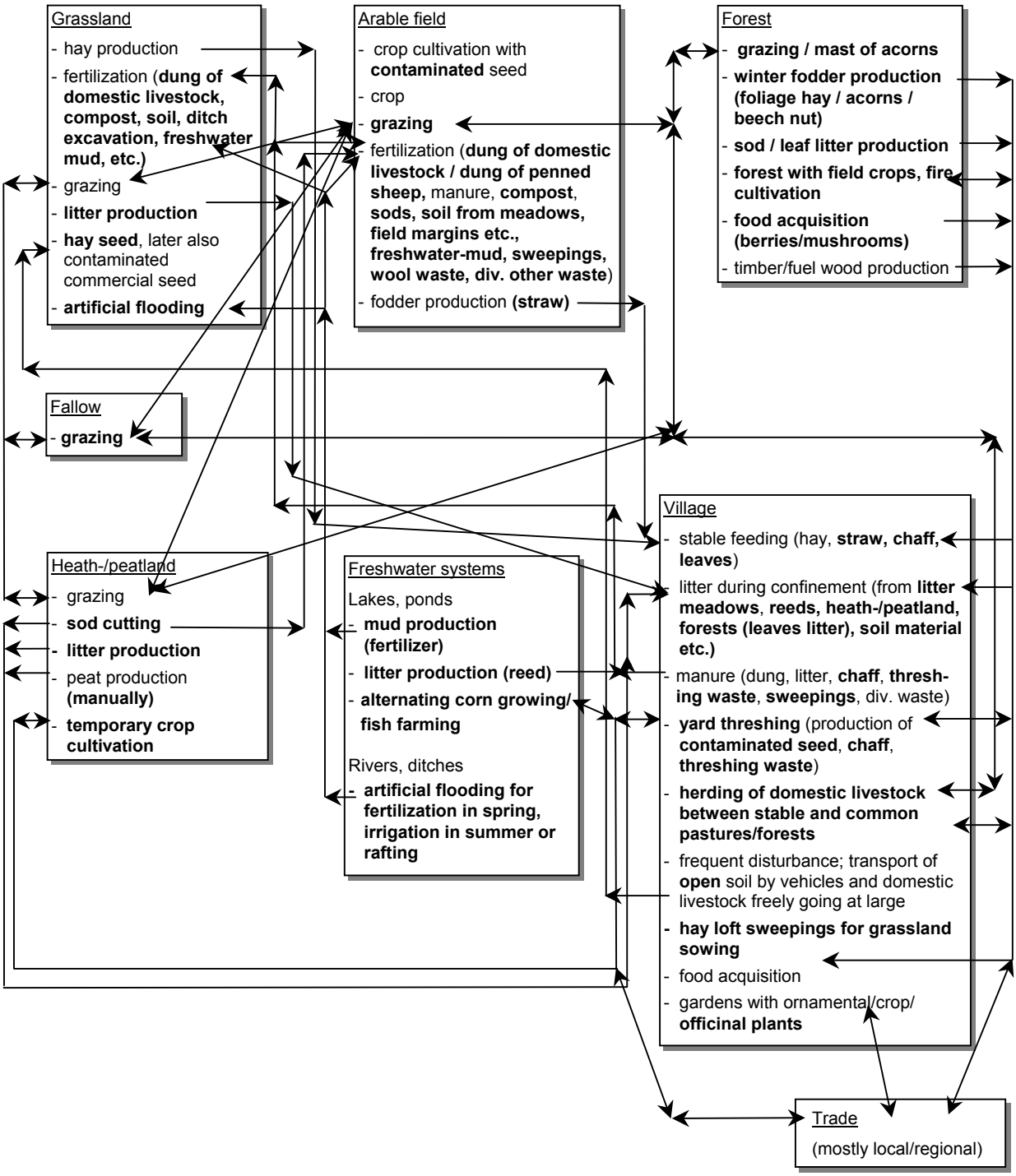

Fig. 2.3 Processes in the historical man-made landscape relevant for dispersal and reproduction. Bold: forms of farming which got lost today; arrows: direction of dispersal.

Today, most of these connections are more or less interrupted (Fig. 2.4). Since the end of the 18th century, for example, the alternating arable field-fallow or pasture management was replaced by rotational field management where the period of abandonment was replaced by the cultivation of root and oil-seed crops, flax, hemp and dye plants (BURRICHTER et al. 1993). Since that time the dispersal of diaspores between arable fields and other habitats occurs only at a very low level or not at all. The same accounts for dispersal processes in the context of other alternating management practices or the big variety of pasturages, which have been vanished during the last two centuries. In total, the predominantly regional dispersal between different habitats has tremendously decreased. If there are any processes left, at least the quantities of diaspores dispersed between habitats are reduced.

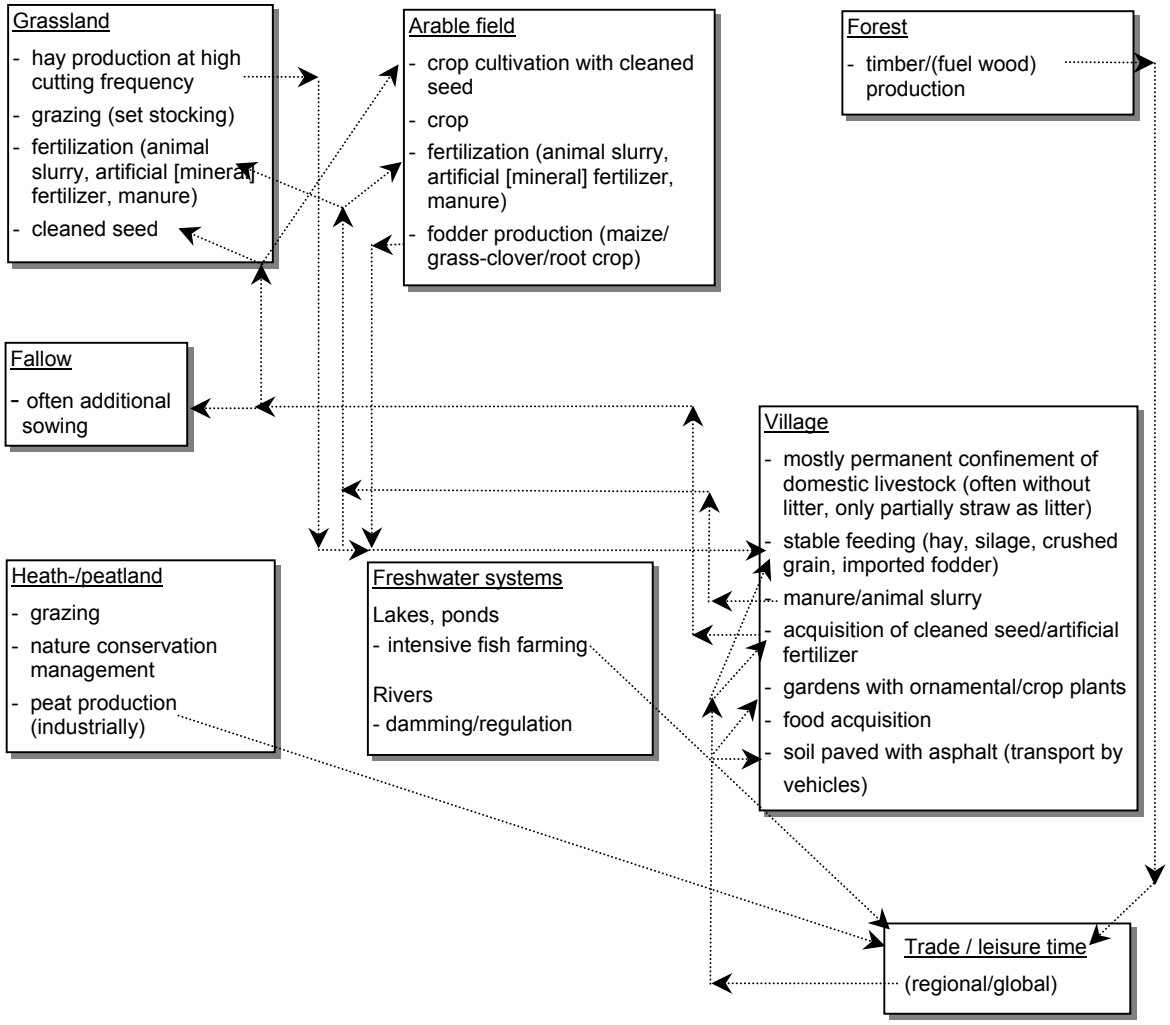

Fig. 2.4 Processes in the present man-made landscape relevant for dispersal.

Arrows: direction of dispersal; dotted line: reduced dispersal relevance compared to the historical man-made landscape.

Table 2.7 Diaspore content of mud adhering to cars (SCHMIDT 1989).
x: species typical for road side vegetation in Germany; xx: species occurring on the road sides in the study area (STOTTELE & SCHMIDT 1988); only the dominant species are mentioned.

	species	number of diaspores	% of total diaspore number (n = 3,926)
xx	*Poa annua*	1,234	31.4 %
xx	*Plantago major*	355	9.1 %
	Epilobium roseum	276	7.0 %
x	*Stellaria media*	231	5.9 %
xx	*Poa trivialis*	221	5.6 %
x	*Urtica dioica*	207	5.3 %
x	*Epilobium ciliatum*	159	4.0 %
x	*Rumex obtusifolius*	145	3.7 %
xx	*Lolium perenne*	70	1.8 %
xx	*Cerastium holosteoides*	68	1.7 %
x	*Sagina procumbens*	59	1.5 %
x	*Prunella vulgaris*	56	1.4 %
xx	*Agrostis stolonifera*	52	1.3 %
x	*Matricaria discoidea*	46	1.2 %
xx	*Poa pratensis* agg.	46	1.2 %
x	*Apera spica-venti*	44	1.1 %
xx	*Dactylis glomerata*	43	1.1 %
x	*Stellaria uliginosa*	42	1.1 %
xx	*Matricaria maritima*	37	0.9 %
xx	*Ranunculus repens*	33	0.8 %
x	*Juncus bufonius*	28	0.7 %
x	*Lamium purpureum*	28	0.7 %
xx	*Taraxacum officinale* agg.	25	0.6 %
x	*Glechoma hederacea*	23	0.6 %
x	*Chenopodium album*	21	0.5 %
xx	*Trifolium repens*	20	0.5 %
x	*Linaria vulgaris*	20	0.5 %
	Salix cf. *triandra*	20	0.5 %
x	*Capsella bursa-pastoris*	18	0.5 %
x	*Juncus effusus*	17	0.4 %
x	*Deschampsia cespitosa*	15	0.4 %
x	*Salix caprea*	15	0.4 %
	Cardamine flexuosa	12	0.3 %
xx	*Holcus lanatus*	10	0.3 %
	34 species (27.4 % of all 124 documented species)	3,696	94.1 %

In contrast to the decrease of dispersal processes depending predominantly on traditional forms of agriculture, trade and traffic tremendously increased during the last decades, acting as important dispersal vectors of modern times (see above, DI CASTRI 1989). Trade in particular causes the dispersal of adventive and weedy species (THELLUNG 1915; SALISBURY 1964; BONN & POSCHLOD 1998a) which establish on heavily or often disturbed sites. Only 385 of more than 12,000 plant species which were introduced in the Central European flora by trade established,

228 of these (mostly cultivated plants) in semi-natural vegetation types, such as *Onobrychis viciifolia* in calcareous grasslands (KOWARIK & SUKOPP 1986; LOHMEYER & SUKOPP 1992). However, agricultural practices were responsible for the dispersal of the species within the landscape. Today, traffic by motor vehicles and railway is also known as a very important dispersal vector. WACE (1977) found 18,566 diaspores from 259 plant species in the mud of a motorcar-washpark in Canberra, Australia. However, SCHMIDT (1989) showed that most of the species dispersed by cars are growing on the road sides (Table 2.7). That means that dispersal by traffic only occurs along roads. This was also shown along railway lines (SUOMINEN 1979). Consequently, this dispersal vector cannot substitute the vectors and processes of the traditional man-made landscape.

Implications for restoration management

Until recently, dispersal was not discussed as a limiting factor for the viability of plant populations (MÜLLER-SCHNEIDER 1977, 1986; VAN DER PIJL 1982; MURRAY 1986; FENNER 1992). However, with the increasing fragmentation of natural and semi-natural habitats in the man-made landscape, especially in Central Europe, dispersal was realized as an important key factor for the survival of fragmented plant populations (OPDAM 1990). Since the metapopulation concept was transferred into plant ecology to interpret plant population dynamics and to analyse the viability of plant populations, dispersal is also discussed on a landscape ecological basis (SILVERTOWN & LOVETT-DOUST 1993; POSCHLOD 1996; POSCHLOD et al. 1996a). Whereas once the discussion was very conservative and dispersal was discussed on the basis of the plants' own traits, e.g. wind dispersal (MCCARTNEY 1990; VERKAAR 1990) it became more and more obvious that in the man-made landscape processes and vectors which are combined with different land use practices are the key for the dispersability of plants (BONN & POSCHLOD 1998a,b; POSCHLOD & BONN 1998). The review on the different land use practices showed that there is a dramatic change and loss of dispersal processes and vectors in our man-made landscape since the last century. The processes and vectors in a landscape with traditional land use forms did not only maintain a permanent diaspore flow between the same habitats but also between most of the different habitats used by man.

Besides pollination (KWAK et al. 1998), dispersal is another important factor for the level of gene flow between populations and individuals (OOSTERMEIJER et al. 1996) and, therefore, is an important key for the long-term survival of plant populations not only in fragmented plant populations but also for non-fragmented populations, especially those of small size. Consequently, it is important that management and restoration practices in nature conservation include this knowledge in future practices. If dispersal processes cannot be restored it is clear that any efforts in restoration management with the goal to provide new habitats for locally or even regionally extinct species will be at odds, except if the species were able survive in the diaspore bank. If this is not the case, dispersal processes have to be simulated or replaced by other ones (POSCHLOD et al. 1998) which can include the artificial reintroduction of species as well (TRÄNKLE & POSCHLOD 1995; BIEWER & POSCHLOD 1996).

Chapter 3 »Diasporus« – a database for diaspore dispersal – concept and applications in case studies for risk assessment

Abstract

Although diaspore dispersal is one of the fundamental processes in a plants' life and therefore an important aspect in the context of biological risk analysis, no comprehensive database on this feature existed to this date. To overcome this deficit, the database »Diasporus« was developed, containing data on documented dispersal modes and standardised species related traits relevant for seed or diaspore dispersal.

Basic steps in the development of the database included the classification of different modes of dispersal and the classification of plant or diaspore traits relevant for dispersal between populations as well as to suitable but so far unoccupied habitats ("long-distance dispersal"). Most existing classifications show a more or less strict combination of the dispersal mode and morphological characters of the diaspore or plant. In contrast, the classification of »Diasporus« is exclusively based on different dispersal vectors, while diaspore morphology is viewed as one of several factors affecting the dispersal potential of plant species. Besides single dispersal relevant traits of the adult plant or the diaspore (time and period of dispersal, diaspore production per plant, releasing height, occurrence of xero- or hygrochasy, diaspore size, shape, weight, specific gravity and morphology), indicator parameters, aggregating various morphological traits, were considered in the classification of dispersal relevant traits. Standard methods for the calculation of these indicator parameters (terminal velocity, buoyancy, attachment capacity and digestion resistance) are proposed.

The database consists of several linked tables, containing the traits of the species studied, the methods used in a dispersal experiment or in determining a certain dispersal relevant trait, and the references for the citation of the studies. The structure of »Diasporus« allows all possible combinations of data included in the reference-, methods- and species-tables. Some examples for the application of the database in the context of viability analysis or risk assessment in plants are illustrated.

Introduction

Diaspore dispersal is one of the fundamental processes in the life cycle of each plant. As early as the 19th century dispersal was recognized to be a vital factor to escape from the intraspecific competition of the parent plant and siblings as well as to colonize suitable habitats which have not yet been occupied ("... schlecht würde es um die Existenz des Pflanzenreiches in seinen einzelnen Arten stehen, wenn die Nachkommen eines jeden Individuums in unmittelbarer Nähe dieses aufschiessen müssten; dieselben würden sich nicht nur den Raum, sondern auch die Nahrung streitig machen und bei andauernder Inzucht allmälig ganz in ihren folgenden Generationen zu Grunde gehen, ohne dass die nun leere Stätte von anderen erreicht und so wieder bevölkert werden könnte ..."*; HILDEBRAND 1873). Due to the tremendous changes in our landscape, which include fragmentation and isolation of species-rich natural and semi-natural habitats, dispersal – in space as well as in time – has again become an important topic in ecological research in the last decade (OPDAM 1988; PRIMACK & MIAO 1992; BAKKER et al. 1996; POSCHLOD et al. 1996a, 1998; THOMPSON et al. 1997; BONN & POSCHLOD 1998a,b; POSCHLOD & BONN 1998). OPDAM (1990) for example considered dispersal in space as the key to survival in fragmented populations. According to the "species pool-concept", dispersal capacity represents an important limiting factor for the species richness of habitats (PÄRTEL et al. 1996; ZOBEL 1997; ZOBEL et al. 1998). Therefore, it is obvious that the individual dispersal potential of plant species has to be considered as a basic element of population viability analysis (MENGES 2000) or "biological risk analysis" (e.g. HUSBAND & BARRETT 1996; POSCHLOD et al. 1996c, 1999, 2000).

Until now, however, a database on dispersal characteristics has been missing, although it might be a basic tool in order to find out whether a certain trait is generally correlated with a rare or threatened state of a plant species or even with the species richness of habitats. There are several reasons why the creation of such a database has not been attempted earlier. Despite the fact that many studies on diaspore dispersal and dispersal relevant traits have been carried out over decades (e.g. BEAL 1898; PRAEGER 1913; HEINTZE 1915; SCHMIDT 1918; RIDLEY 1930; HARMON & KEIM 1934; DANSEREAU & LEMS 1957; BURROWS 1973; MÜLLER-SCHNEIDER 1977, 1986; VAN DER PIJL 1982), and numerous data on the different modes and vectors of dispersal for a certain plant species have been documented during this time, data were never systematically collected. In many cases, it is not even clear, whether any direct measurements were involved or whether the presented data are the result of simple assumptions. Furthermore, existing data are often scattered or not available.

To close this gap we have started to construct a database containing standardised species related traits relevant for seed or diaspore dispersal. The term "diaspore" is used because a dispersal unit may not only be a seed, but also a fruit or, as in the case of ferns, a spore or even a vegetative propagule such as a bulb, a part of the rhizome and so on (POSCHLOD & JACKEL 1993).

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* "... the individual species of the vegetable kingdom would greatly suffer if the descendants of each individual were to grow tall in the immediate vicinity of the parent plant. The self-same plants would not only compete for space but also for nutrition. And as continuous inbreeding would cause them to slowly perish within generations, there would be no chance of others reaching the abandoned spot to recolonize it ..."

The use of this term, which is not very common in the English literature, stresses the additional need of creating a ”common language” (WEIHER et al. 1999) in ecology. Therefore, another goal of this database is to provide ”common terms” for dispersal ecology. Finally, a last aim is to show exemplary applications of the database with respect to viability analysis or risk assessment for single species or even all species of a community.

Classification and concept of the database

Prior to constructing the database, two questions were addressed:

1. How to classify the different modes of dispersal?
2. Which traits of the plant or diaspore have to be considered as relevant for these different modes of dispersal, affecting either the mode of dispersal itself or the dispersal distance?

Classification of dispersal modes

In the past, several dispersal classifications have been developed (e.g. DANSEREAU & LEMS 1957; MÜLLER-SCHNEIDER 1977; LUFTENSTEINER 1982; VAN DER PIJL 1982; GRIME et al. 1988; JENNY 1991; FREY & HENSEN 1995). However, apart from the fact that some of these classifications ignore certain modes of dispersal, such as dispersal by water (see LUFTENSTEINER 1982) or all kinds of dispersal by human activity (e.g. LUFTENSTEINER 1982; VAN DER PIJL 1982; JENNY 1991), the existing classifications fail to clearly separate the mode of dispersal from the dispersal relevant morphological characters of the plant or diaspore. The simple deduction of in most cases one single dispersal mode from morphological traits of a diaspore makes it obvious that for decades dispersal studies have focused on the observation of those dispersal events expected by diaspore morphology (see also BERG 1983); many classified dispersal modes have probably never even been measured or observed in the field.

Although morphological characters of the diaspore provide some information on their dispersal potential, a strict allocation of a certain diaspore morphology to only one dispersal mode may result in a completely wrong interpretation of the real dispersal potential of plant species (HOWE & SMALLWOOD 1982; BONN & POSCHLOD 1998a). Many grassland species, for example, which are classified according to their diaspore morphology as poor, auto- or semachorous dispersers (e.g. LUFTENSTEINER 1982; MÜLLER-SCHNEIDER 1986), can be dispersed in high quantities over large distances by domestic livestock (e.g. FISCHER et al. 1995, 1996). Various neophytes of Central Europe, like *Impatiens glandulifera* and *Reynoutria japonica* or *R. sachalinensis*, represent obvious examples of this discrepancy between the dispersal mode deduced from diaspore morphology and the dispersal potential as well as the species distribution actually studied in the field. Diaspores of *Impatiens glandulifera* are dispersed over a few meters via ejection by the parent plant (LHOTSKÁ & KOPECKÝ 1966), while *Reynoutria* spreads predominantly vegetatively via rhizomes (SUKOPP & SUKOPP 1988; HARTMANN et al. 1995). According to this classification, the dispersal potential of these neophytes is limited to short distances. Nevertheless, they have spread very rapidly in Europe – a fact that obviously indicates

the existence of alternative ways of dispersal. In these cases, long-distance dispersal has not only been effected by human activity (via cultivation, garden throw-outs), but also hydrochorous by flowing water (LHOTSKÁ & KOPECKÝ 1966; SUKOPP & SUKOPP 1988; HARTMANN et al. 1995), which is reflected by the frequently observed occurrence of these species along river banks.

Table 3.1 Classification of dispersal modes based on the dispersal vector.

The grey coloration indicates a high potential for long-distance dispersal (sensu HANSSON et al. 1992); barochory (dispersal by gravitation) is excluded from this system, because the distinction between anemochorous and barochorous dispersal is gradual.

	dispersal mode			**dispersal by ...**
1.	**Autochory**	a.	ballochorous	ejection by the parent-plant
		b.	blastochorous	active deposition by the parent plant
		c.	herpochorous	creeping hygroscopic hairs of the diaspore
2.	**Semachory**		semachorous	swaying motion of the parent plant caused by external forces (wind, ...)
3.	**Anemochory**		anemochorous	wind
4.	**Hydrochory**	a.	ombrochorous	ejection caused by falling rain drops
		b.	nautochorous	water (swimming on water-surface)
		c.	bythisochorous	flowing water (on the ground)
5.	**Zoochory**	a.	myrmecochorous	ants
		b.	ornithochorous (epizoo-, endozoo-, dysochorous)	birds (on the body surface, via ingestion, via transport for nutrition)
		c.	mammaliochorous (epizoo-, endozoo-, dysochorous)	mammals (on the body surface, via ingestion, via transport for nutrition)
		d.	anthropochorous	man (on the body surface, via ingestion, via transport for nutrition*)
		e.	others (epizoo-, endozoo-, dysochor)	other animals (snails, earthworms, ...) (on the body surface, via ingestion, via transport for nutrition)
6.	**Hemerochory**	a.	agochorous	human action (trade, traffic, litter, soil movement, ...)
		b.	speirochorous	seeds, as impurities
		c.	ethelochorous	commercial seeds/cultivation

*: this dispersal mode includes only accidental dispersal, comparable to that by animals.

These examples illustrate that diaspores may potentially be dispersed by different vectors (“polychory”). However, the actual dispersal mode also depends on the structure and ”dispersal infrastructure” of a certain landscape, which includes static as well as moving corridors (POSCHLOD et al. 1996a) provided mainly by the different types of land use (BONN & POSCHLOD 1998a,b; POSCHLOD & BONN 1998; POSCHLOD et al. 1998, see chap. 2). Therefore, our classification of dispersal modes (Table 3.1) is based on dispersal vectors.

Classification of plant traits relevant for diaspore dispersal

Various traits of the dispersal unit or the whole plant may affect the “dispersability” by a certain vector. To assess the dispersal potential of a diaspore, we had to decide which traits should be considered as relevant for the different dispersal modes, affecting either the mode of dispersal or the dispersal distance. During the last years, there have been only few approaches, which assess “dispersability” by relating certain plant or diaspore traits to a single dispersal mode (e.g. GREENE & JOHNSON 1989; HODKINSON & THOMPSON 1997; THOMPSON et al. 1999; WEIHER et al. 1999). However, until now, there has been no proposal for a comprehensive system that associates plant and diaspore traits with the dispersal potential of plant species, taking into account all possible dispersal vectors.

Therefore, we propose such a system, which is presented below as a first approach (Table 3.2). This system focuses on dispersal modes characterised by the potential for dispersal between populations or to suitable but as yet unoccupied habitats (”long-distance dispersal” sensu HANSSON et al. 1992). Although dispersal modes restricted to a potential for short distance dispersal within an existing population are important for the structure and dynamics of local populations and are, therefore, important determinants of a metapopulation (HUSBAND & BARRETT 1996), we decided to exclude these dispersal modes from the classification. Apart from keeping the database as simple as possible, the main reasons were:

1. The database was developed to analyse a species’ viability in a certain landscape. In this context, we do not consider single populations but a set of populations on the landscape level. Therefore, long-distance dispersal becomes more relevant.

2. Since there are always several possibilities for a species to disperse within a local population, a population will rarely suffer from restricted ”short-distance dispersal”. In a landscape that lacks adequate vectors for long-distance dispersal, species will invariably be dispersed over short distances, regardless of their different dispersal potentials. The only factor limiting short-distance dispersal is the number of produced diaspores. Therefore, diaspore production is a simple but adequate indicator of the short-distance dispersal capacity (Table 3.2).

The following section briefly explains the plant and diaspore traits considered outlining their relevance for the different dispersal modes mentioned.

Time and period of dispersal affect the dispersal potential of all dispersal modes by determining whether diaspores are released when a specific dispersal vector is available, e.g. grazing animals or water during a flooding event. Since height and density of vegetation, litter fall and wind speeds change during the season, time and period of dispersal may affect their wind dispersal potential as well (SHELDON & BURROWS 1973; SHARPE & FIELDS 1982; JOHNSON 1988).

The higher the **number of diaspores produced**, the more variable the dispersal distances and the larger the area that can be covered via a certain dispersal vector. Furthermore, seed input per area is increased. Finally, higher seed numbers increase the chance of at least few seeds extending the end or "tail" of the dispersal curve and leading to higher absolute dispersal distances (HARPER et al. 1970; DIRZO & DOMINGUEZ 1986; WILLSON 1993b). Again, all modes of dispersal are affected by this trait.

The **releasing height** (height of the infructescence) determines not only whether a diaspore is within reach for a certain vector (e.g. an animal, certain machines or in some cases also water) but also the trajectory and, therefore, the dispersal distance in the case of anemochory.

The occurrence of **xero- or hygrochasy**, that means seed shed occurs exclusively in dry or wet weather conditions, is especially important in the case of anemochorous dispersal. Since weather conditions with low humidity typically offer better flying conditions, drought-dependent shed of diaspores (xerochasy) favours higher dispersal distances. In contrast, a water-dependent opening of the capsules (hygrochasy) guarantees that diaspores are shed during rainy weather situations which is typical in several species dispersed by water (KOHLERMANN 1950; MÜLLER-SCHNEIDER 1977; VAN DER PIJL 1982).

Diaspore size and shape may affect the ability of a diaspore to survive the passage of the digestive tract of animals – small, spherical diaspores show higher survival rates, because they are less susceptible to damage by chewing and often pass the digestive tract faster (LENNARTZ 1957; GARDENER et al. 1993a). Additionally, a small diaspore size was shown to be favourable for epizoochorous dispersal in the coats of wild boars (MROTZEK et al. 1999). Predominantly small seeds were also found in soil samples collected from the hooves of sheep grazing on calcareous grassland (FISCHER et al. 1995). Furthermore, size and shape also affect the ability to escape seed cleaning – diaspores with a size and shape similar to crop seed (SCHNEIDER et al. 1994) or of very small size (HOLZNER 1981) pass the traditional forms of seed cleaning.

The shape of a diaspore can be described as a figure by measuring its length, width and height and calculating the approximation to a sphere (THOMPSON et al. 1993; BAKKER et al. 1996).

Diaspore weight and specific gravity are also of importance for different modes of dispersal. They may, for instance, affect the capacity to be dispersed endozoochorously, because they influence the retention time in the digestive tract (EHLE & STERN 1986; GARDENER et al. 1993a). Furthermore, epizoochorous, nautochorous (VAN DER PIJL 1982) as well as anemochorous dispersal (GREENE & JOHNSON 1993) or dispersal by impure crop seed (BARRETT 1983; MCDONALD & SMITH 1990) are influenced by the weight and specific gravity of the dispersule.

Diaspore morphology, such as a surface with hooks or a hard seed coat, affects all modes of zoochorous dispersal. In the case of epizoochorous dispersal, the existence of hooks, awns or

bristles favours attachment to the fur (SHMIDA & ELLNER 1983; FISCHER et al. 1996; KIVINIEMI & ERIKSSON 1999; MROTZEK et al. 1999), whereas smooth diaspores are predominantly transported in the hooves (FISCHER et al. 1995; STENDER et al. 1997). Both, a hard seed coat and a smooth diaspore surface increase the chance to survive the passage of the digestive tract (LENNARTZ 1957; ÖZER & HASIMOGLU 1977; ÖZER 1979). Although wind may function as dispersal vector for all kinds of diaspores, the existence of a pappus or wings will favour higher dispersal distances. Moreover, morphology may affect the ability to float – for example due to a water-repellent diaspore surface – and in some cases, it affects the dispersal by impure crop seed (speirochory).

For several dispersal modes it is possible to measure **indicator parameters**, which aggregate various morphological traits of the diaspore (Table 3.2). Terminal velocity, attachment capacity, digestive tolerance and buoyancy are the result of a certain diaspore size and shape, certain morphological traits of the diaspore, like plumes in the case of terminal velocity, and specific gravity. These indicator parameters allow a more precise and easier assessment of the dispersal potential by a certain vector. Additionally, these aggregated parameters may facilitate the modelling of dispersal processes, since they can be taken as direct input parameters for mechanistic dispersal models (ANDERSEN 1991). However, data for most of these aggregated parameters are very scarce or even entirely missing. In this respect, a standardisation of the methods employed to obtain these indicator parameters is an important aspect for future research.

Terminal velocity of a diaspore is one of those indicator parameters, which have already been measured in a standardised way for a larger amount of species (e.g. ASKEW et al. 1997; JONGEJANS & SCHIPPERS 1999; TACKENBERG 1999; THOMPSON et al. 1999). In combination with the initial releasing height of the diaspore, it can be used as a measure for the wind dispersal potential of a plant species (e.g. GREENE & JOHNSON 1989; ANDERSEN 1991; JONGEJANS & SCHIPPERS 1999; TACKENBERG 1999).

A relatively precise indicator parameter for the epizoochorous dispersal potential of plant species is to measure the **attachment capacity** of diaspores in the fleece or fur of animals. Attachment capacity can be measured as the percentage of attached diaspores per time unit after standardised diaspore application and simulated animal movement. Although dispersal via mud on the feet or hooves of animals (e.g. DARWIN 1859; KERNER 1898; FISCHER et al. 1995) may also play an important role in long-distance dispersal, this mode of dispersal seems more or less unpredictable by morphological traits of the plants.

Another standardised method is used to measure the **potential to survive the passage of the digestive tract** in order to classify diaspores after their potential for endozoochorous dispersal. The survival rate is measured in vitro (see SIMAO NETO & JONES 1987; GODÍNEZ-ALVAREZ & VALIENTE-BANUET 1998).

Finally, the **buoyancy** of a diaspore characterises the dispersal potential in running or standing waters. It can be measured as the proportion of diaspores still floating after defined periods of time (e.g. POSCHLOD et al. 1996b; BILL et al. 1999).

Table 3.2 Diaspore and plant traits relevant for long-distance dispersal (sensu HANSSON et al. 1992).

The **black cells** indicate a high importance of a trait for a certain mode of dispersal, the **light grey cells** a minor importance or an importance only for some species, traits emphasized by a **dark grey colour** can be summarized by indicator parameters.

Traits	Collected data	Anemo-chory	Zoochory			Hydrochory		Hemero-chory
			epizoo-	endozoo-	dyso-	nauto-	bythiso-	speiro-
I. Plant-traits/traits of the infructescence:								
a. time and duration of diaspore spreading	begin, end [month(s)]							
	duration [month(s)]							
b. diaspore production per plant or ramet	[number]							
c. seeds per diaspore	[number]							
d. releasing height	[m, classes]							
e. xerochasy/hygrochasy	categorization (yes/no)							
II. Morphological traits of the diaspore:								
a. diaspore size & shape	length [mm]							
	width [mm]							
	height [mm]							
	shape [calculated number]							
b. diaspore morphology	categorization (burr, plumed, hard-coated ...)							
c. diaspore weight/ specific gravity	[mg]/ [g/ml]							
III. Indicator parameters:								
a. terminal velocity	[m/s]							
b. attachment capacity (different fur types)	[percent/time unit]							
c. tolerance for passing the digestive tract (survival rate)	[percent]							
d. buoyancy	[days]							

Problems may arise when plant traits are used to estimate the dispersal potential of human actions (hemerochory), although this mode of dispersal strongly affects the composition and species richness in man-made habitats (POSCHLOD et al. 1996a; BONN & POSCHLOD 1998a,b; POSCHLOD & BONN 1998, see chap. 2). A relationship between hemerochorous dispersal and plant traits is obvious in the case of dispersal by impure crop seed (speirochory). In contrast, ago- and ethelochorous dispersal do not seem to be affected by any traits of the plant or diaspore that can be categorised. Although HODKINSON & THOMPSON (1997) found that in the case of dispersal by transport of topsoil, cars and horticulture or by garden throw-outs dispersal is associated with certain traits of the plant and diaspore, ago- or ethelochorous dispersal does not directly depend on any plant or diaspore trait. Especially agochorous dispersal includes a great variety of different ways of dispersal – these are, however, unselective with respect to plant or diaspore traits.

Other problems concern traits, which affect the dispersal potential, but are too difficult to measure for a large amount of species. For example, dispersal potential by wind and epizoochorous dispersal are affected by the ”resistance” of a diaspore to detach itself from the parent plant (KOHLERMANN 1950; FALINSKI 1972; JOHNSON 1988; GREENE & JOHNSON 1989; 1992). In the case of anemochorous dispersal, for example, a high ”detachment resistance” restricts diaspore release to times of sufficiently strong wind conditions. In relation to other diaspores this trait may compensate for a higher terminal velocity and lead to similar or even higher dispersal distances (JOHNSON 1988). Due to the changing ”detachment resistance” of a diaspore during a vegetation period, however, the forces necessary to detach a diaspore may vary considerably (AUGSPURGER & FRANSON 1987).

2.3 Conception of the database

The database »Diasporus« consists of several linked tables (Fig. 3.1), which can be summarised under three categories:

- The »data tables«:

  They contain the traits of the species studied. On the one hand, this concerns the mode of dispersal (Table 3.1) that has been documented either in own experiments or by other authors. Some further information can be added, for example the animal species in the case of zoochorous dispersal or the documented weather situation and wind speed with regard to anemochorous dispersal. On the other hand, a separate data table was created for each of the dispersal relevant traits of the plant or diaspore shown in Table 3.2. Apart from measurements conducted by the authors themselves, these data have been taken over from other databases (HODGSON et al. 1995; KLEYER 1997) and several publications. The data collected consist of absolute measured data, classified data and various prescribed categorisations such as diaspore morphology and type (see Table 3.2). Each trait is documented as a single record in the database, e.g. we found terminal velocities for *Taraxacum officinale* in 16 different studies, which are stored as separate records. The variation of traits within a single study can be estimated by using the minimum, mean and

maximum values (Fig. 3.1). In the case of plants producing heteromorphic diaspores (SORENSEN 1978; MCEVOY & COX 1987; REDBO-TORSTENSSON & TELENIUS 1995), different diaspore types are considered separately.

The data tables are linked by the species' name, with the nomenclature currently following OBERDORFER (1994). When linking to other databases, however, the fact that a reliable and automatic synonymisation is still missing may pose serious problems (THOMPSON et al. 1997).

- The table »methods«:
  This table contains the standardised description of the dispersal experiment(s) and the methods to determine the different traits (e.g. buoyancy by floating experiments). It includes information on the vegetation type the experiments were conducted in, tests for germination or viability and on the location the study took place.
- The table »reference«:
  This table cites the literature, in which a dispersal-study or the measuring of a plant or diaspore trait is described.

The structure of »Diasporus« allows all possible combinations of data included in the data-, methods- and reference-tables. Queries may, for instance, relate only to definite dispersal modes obtained with a certain study-method, to different dispersal modes of a certain plant species or of diaspores with certain characteristics (e.g. a definite size, shape or weight) – or to data on a certain dispersal vector.

Applications and current constraints of »Diasporus«

In the following section, some examples of possible applications of the database in the context of viability analysis or risk assessment in plants are illustrated.

The first example concerns changes in the frequency of plant species on the landscape level (10 x 10 kilometer grid). Specifications on changes in frequency refer to the occurrence of species in a grid as well as the frequency of a species within a certain grid before and after 1945 (HAEUPLER & SCHÖNFELDER 1989; ELLENBERG et al. 1992; see also SCHÖNFELDER 1999). Based on more than 3,500 records, 785 species were examined for dispersal modes with a high potential for long-distance dispersal (see above). If any record documenting the potential for long-distance dispersal was missing, a species was classified as "species without potential for long-distance dispersal". To assess the importance of "dispersability" in the context of risk analysis, we examined the extent to which the occurrence of these species has changed in West-Germany in the last decades in relation to their documented dispersal potential (Fig. 3.2). The correlation of data on dispersability and changes in frequency of plant species (Fig. 3.2) illustrates, that species lacking a high potential for long-distance dispersal show a decrease in frequency more often than species that have the potential for long-distance dispersal. In contrast, the majority of the species without

changes or even with an increase in frequency since 1945 shows a high potential for long-distance dispersal.

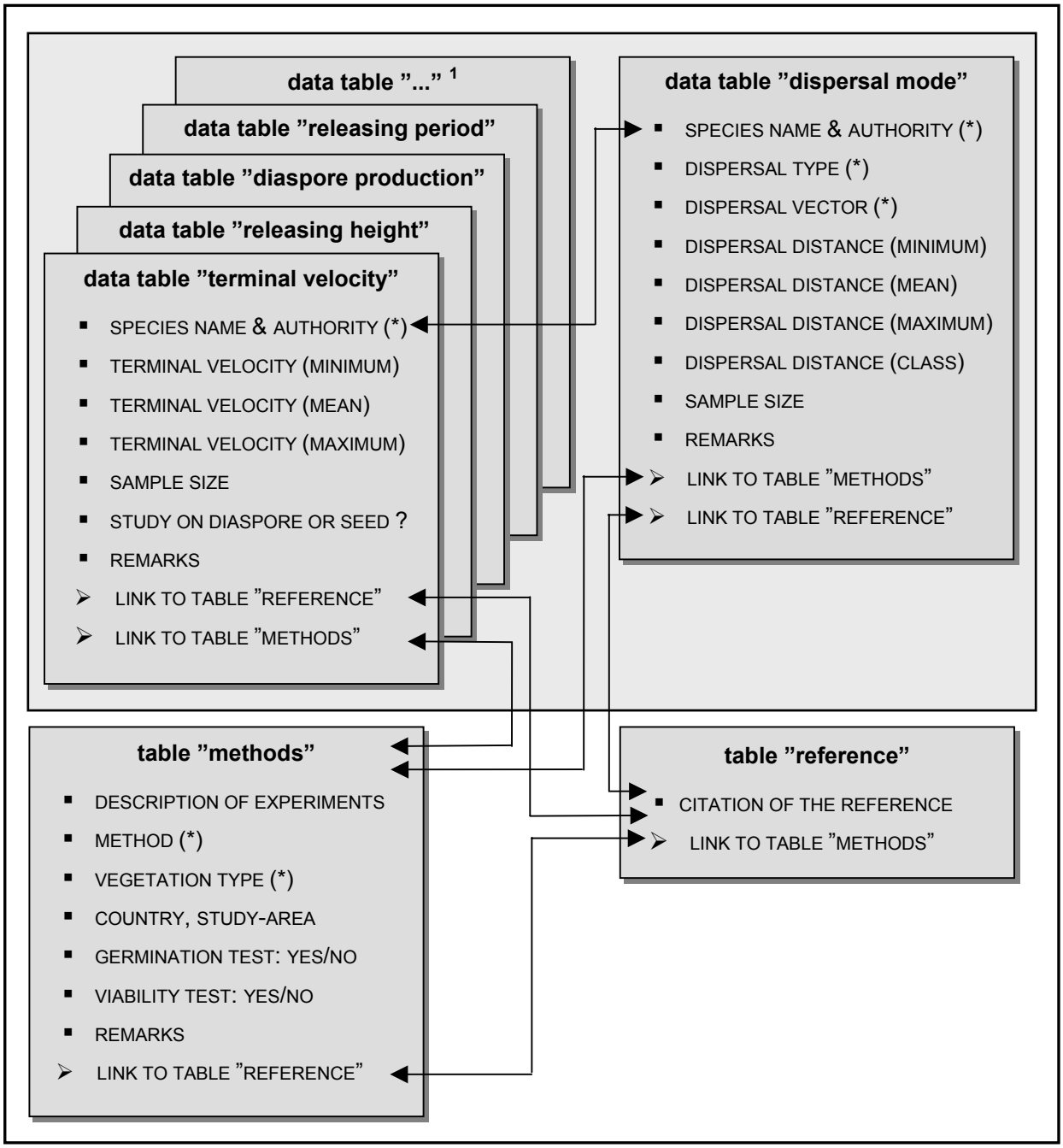


Fig. 3.1 Basic structure of the database »Diasporus«.

(*) : fields with prescribed categorizations in the background; [1]: altogether »Diasporus« includes 13 data tables for the characterization of the dispersal relevant plant or diaspore traits and the different indicator parameters (s. Table 3.2).

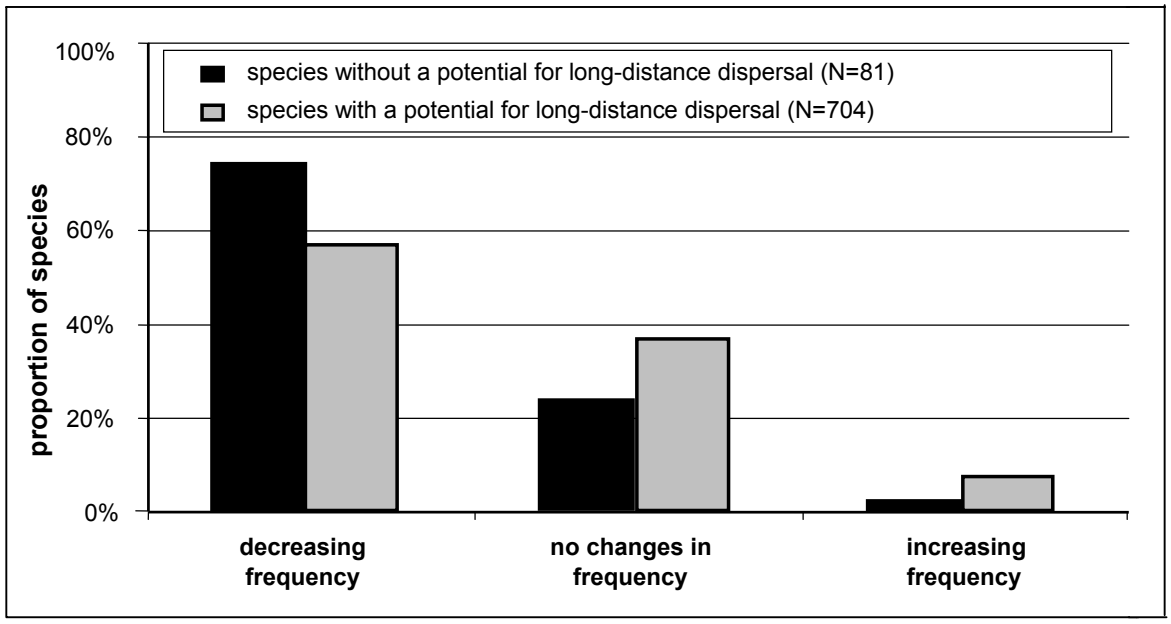


Fig. 3.2 Dispersal potential of 785 plant species in relation to observed changes in their frequency in West Germany since 1945.

The changes in frequency are based on grid based floristic mapping on the landscape level (ELLENBERG et al. 1992).

This relationship between “dispersability” and change in frequency suggests that the potential for long-distance dispersal influences changes in frequency and distribution of a species in the Central European landscape during the last decades. However, it is actually difficult to assess whether this plausible relationship reflects ”reality” or is merely caused by the following problems, some of which have already been mentioned above:

1. Systematic studies on diaspore dispersal are still very scarce and different modes of dispersal have been studied with different intensity. Especially data about epizoochorous dispersal are very rare and many species, which may obviously be transported by an animal, are not mentioned in the literature. For example, in a study conducted by HODGSON et al. (1995), *Chaerophyllum temulum* was classified as barochorous, even though it seems likely that this species has also a potential to be dispersed epizoochorously which is confirmed by existing studies on epizoochorous dispersal. Diaspores of *Anthriscus sylvestri*s, for instance, which are comparable to those of *Chaerophyllum temulum* as regards diaspore morphology and releasing height, have been frequently observed in the fleece of sheep (FISCHER et al. 1996).
2. Furthermore, studies conducted in different habitats are assumed to have focused on certain dispersal vectors. For example, studies on freshwater communities typically examine dispersal by water, neglecting any other dispersal vectors.

One way to overcome these problems is to study the aggregated dispersal relevant traits or "indicator parameters" (Table 3.2) in laboratory experiments using standardised methods and examining a great number of species (see below). Table 3.2 can be used as a rough guide to the plant and diaspore traits relevant for a certain dispersal vector. For more detailed analyses, however, standardised classification methods are needed.

Another example to use the database is the analysis of the different modes by which certain plant species may be dispersed. With the help of such an analysis it is not only possible to retrospectively understand, but also to predict the consequences of changes in land use for certain plant species or all species of a community. Flood-plain meadows, for example, were used as pastures for domestic livestock in former times; usually they were grazed by domestic pigs but sometimes also by other domestic livestock (BEINLICH 1998). Today, the grazing of these wet grassland systems has been abandoned in Central Europe, and many species of these habitats have become rare or even extinct (PHILIPPI 1969; NEBEL et al. 1990; SCHNEIDER-JACOBY 1994). In other parts of Europe, however, these traditional pastures, grazed by cattle, horses and pigs, still exist, e.g. in Croatia along the river Save (BEINLICH 1998).

Correlating the plant species of these grazed flood-plain meadows with existing data on their dispersal potential by different vectors (SCHÖNFELDER 1998), it becomes evident that most of the species may be dispersed by domestic livestock either epi- or endozoochorously and are in consequence able to cover large distances via these mobile vectors (Fig. 3.3). However, if grazing management would be abandoned, as it has happened in many areas of Central Europe, zoochorous dispersal by domestic livestock would be no longer existing. The ensuing change in dispersal processes and distances that can be covered, might be crucial especially for those plant species, which are not dispersed by wind or water over long distances and do not persist in the diaspore bank for several years or decades.

If regular flooding would be prevented as well, as it has happened in many other landscapes, species dispersed nautochorously are predicted to similarly loose their ability to cover long distances by running water (Fig. 3.3).

These examples suggest that the decline or even extinction of typical species of these flood-plain meadows may not only be caused by the loss of habitats and lack of disturbances (PHILIPPI 1969; MICKLICH et al. 1996; BEINLICH 1998), but also by the increasing habitat fragmentation resulting from the loss of dispersal processes between populations formerly effected by a "dynamic moving infrastructure" (POSCHLOD et al. 1996a).

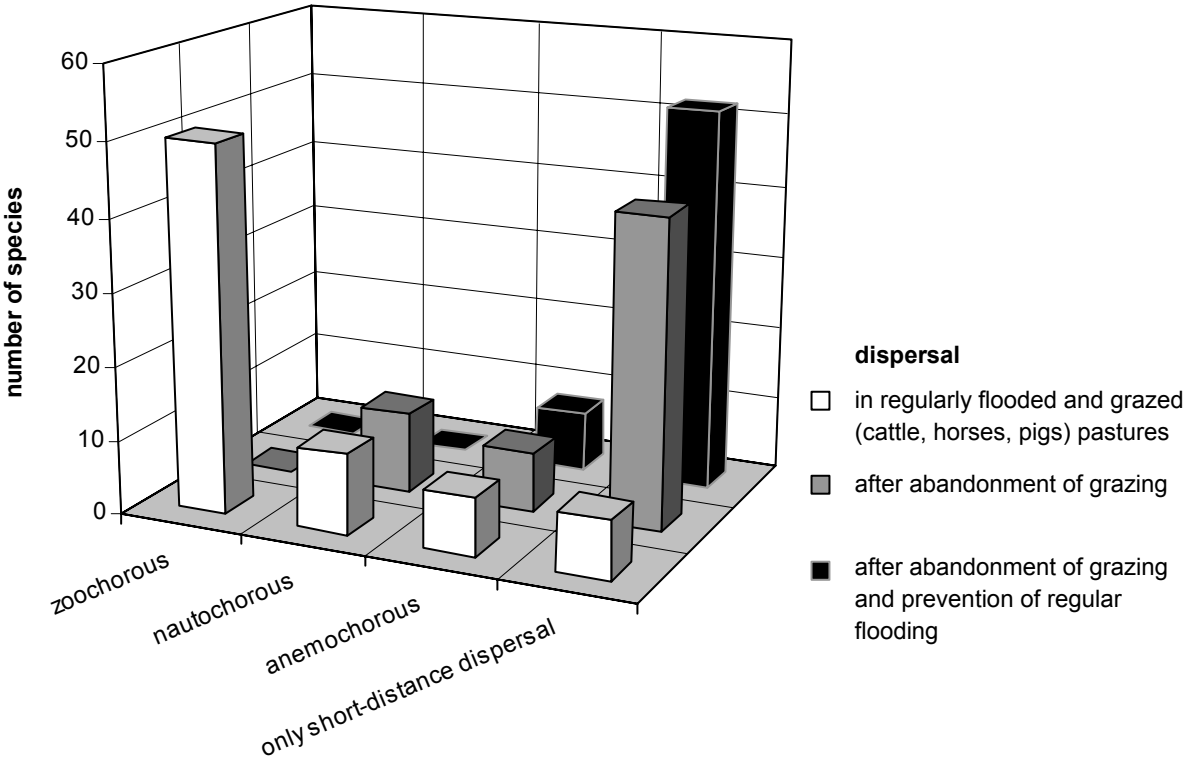


Fig. 3.3 Dispersal spectra of plant species of the flood-plain meadows along the river Save (Croatia) caused by changes in the "dispersal infrastructure".

Data from SCHÖNFELDER (1998); species are considered several times, if different modes of dispersal have been documented (e.g. anemo- and nautochorous dispersal).

In a third example, the application of the data in the context of modelling is presented. Dispersal curves describing the probability of covering a certain distance are very difficult to measure experimentally. It has been suggested that the application of mechanistic dispersal models might result in a better approximation of dispersal curves (e.g. OKUBO & LEVIN 1989; ANDERSEN 1991). In spite of such models, however, it remains difficult to predict rare dispersal events at the end or the "tail" of the leptokurtic dispersal curve (PORTNOY & WILLSON 1993; STRYKSTRA et al. 1998; BULLOCK & CLARKE 2000). In the case of holocene migration of certain plant species, for instance, species distribution calculated by diffusion models by far surpassed their actual distribution in the present landscape (CAIN et al. 1998). Therefore, a more accurate parameter for the quantification of diaspore dispersal on the basis of such models is the distance within which the majority of diaspores (e.g. 95%) will disperse (STRYKSTRA et al. 1998) or the proportion of diaspores that reach a predefined reference distance.

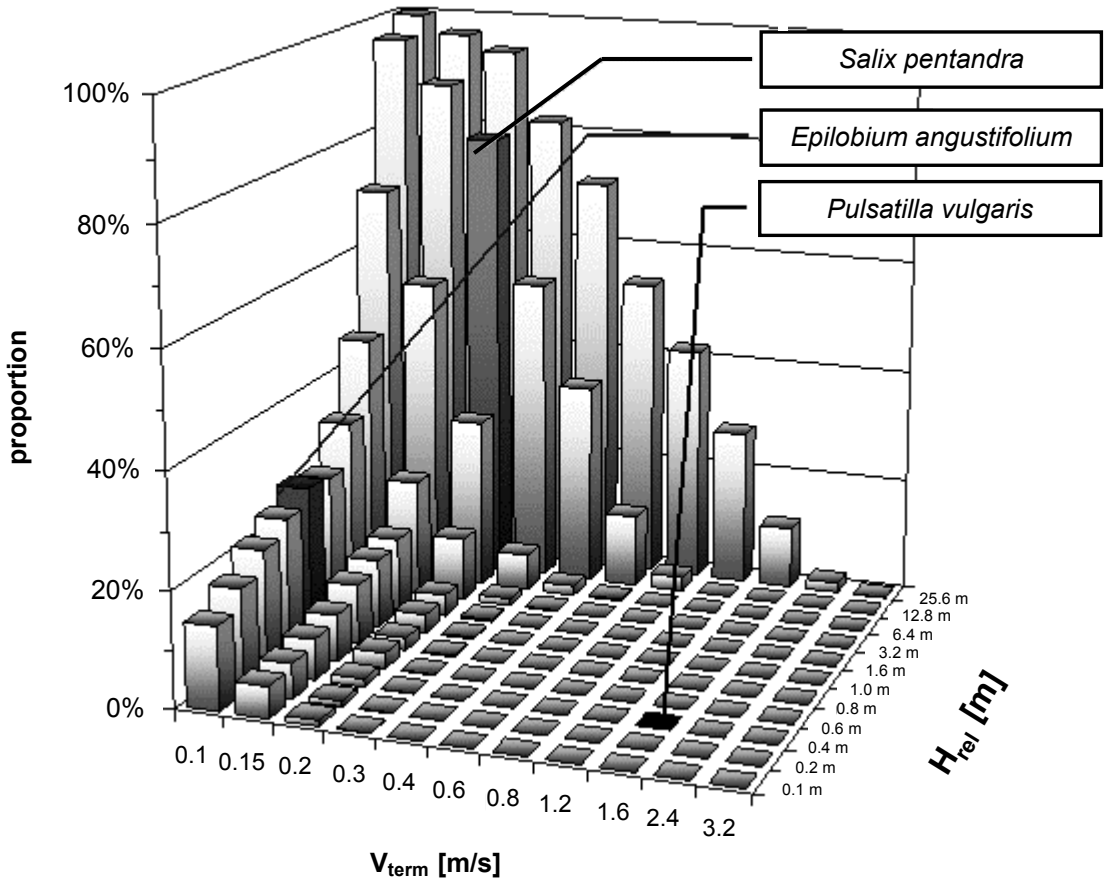


Fig. 3.4 Proportion of diaspores that reach dispersal distances of more than 25 m.

V_{term}: terminal velocity in meters per second; H_{rel}: releasing height of the diaspores in meters. The calculated proportions are valid only for isolated diaspore sources (point source) in a flat landscape in special weather conditions (typical for a labile atmosphere with thermal turbulence; for further details: see TACKENBERG 1999).

For the modelling of anemochorous dispersal, we developed a flight trajectory model (TACKENBERG 1999) in which the species are characterised by the traits ”releasing height” and ”terminal velocity” of the diaspore. With the help of this model landscape related seed shadows and dispersal probabilities can be calculated.

Additionally, the output of this model can also be used to assess the wind dispersal capacity of plant species. Figure 3.4 shows the computed proportion of diaspores that reach distances of more than 25 m in a certain weather situation and in a specific landscape. In the graph, the proportion of diaspores that reach this distance is calculated for combinations of 11 releasing heights and 11 terminal velocities based on 1.000 simulated trajectories for each combination.

In Figure 3.4 three species commonly classified as anemochorous are highlighted. The results from the simulation model indicate substantial differences in dispersal potential among these so-called ”anemochorous” species. In the situation modelled, more than 90 % of the *Salix*

pentandra diaspores reached distances of more than 25 m, whereas none of the 1.000 trajectories calculated for the diaspores of *Pulsatilla vulgaris* resulted in such a distance. This difference in the dispersal potential of various ”anemochorous” species indicates that for many questions related to “dispersability”, the common distinction between ”anemochorous” versus ”non anemochorous” species may often lead to erroneous interpretations.

Therefore, the output of the wind dispersal model was used to develop a more accurate classification of the wind dispersal potential of different plant species. In this model, the absolute value of the computed proportion of diaspores that reach a certain distance varies over magnitudes when changing weather conditions and landscape situations are taken into account, but the relationships between the species remain relatively constant over a wide range of conditions. Therefore, the proportion of diaspores that reach a certain distance can be used to classify the wind dispersal potential of plant species (TACKENBERG 1999; TACKENBERG et al. 2003). Based on this method and the data in »Diasporus«, the wind dispersal potential of more than 300 species was classified. This species specific wind dispersal potential can be used to predict the degree to which a species is threatened by fragmentation processes.

Conclusion

The examples given above show that a comprehensive database on diaspore dispersal may serve as an important tool in the context of risk analysis. However, standardised data on dispersal relevant morphological and especially on aggregated diaspore traits as basic elements of such applications remain very scarce. Additionally, dispersal in space is but one factor for risk analysis in plant species. Therefore it is necessary to standardise the development of other databases which are relevant in this context. A final aim should be a comprehensive database containing all risk-relevant plant traits.

Chapter 4 Assessment of endozoochorous dispersal potential of plant species by ruminants – approaches to simulate digestion

Abstract

Diaspore dispersal via passing the digestive system of ruminants is an important dispersal mechanism in natural as well as cultural landscapes. The assessment of endozoochorous dispersal potential of plant species is therefore crucial in the context of conservation biology and restoration ecology. However, methods to assess endozoochorous dispersal potential do virtually not exist. Since feeding experiments are very elaborate and time consuming, they have been conducted only with few plant species. Therefore, various approaches were tested in order to find an alternative, preferably easy and quick method to assess endozoochorous dispersal potential. The approaches were related to the results of a feeding experiment with 14 plant species fed to cattle and sheep. Mastication, microbial and chemical digestion as the three basic steps of the ruminant digestive system were simulated by in vitro-digestion, simulated chewing followed by an immersion in acid, or imbibition in anoxic liquid and HCl. Although survival after in vitro-digestion showed the best correlations with relative germination after in vivo-digestion, it was rejected as a standard method because of the continuous need of rumen fluid. Mastication, simulated by mechanical stress via an iron stick, followed by an immersion in 0.1 M HCl over 8 hours proved to correlate also strongly with the results of the feeding experiment with sheep. Because sheep provide a more conservative estimate of a plant species survival capacity when passing the ruminants guts, this method is recommended as a suitable standard method to estimate endozoochorous dispersal potential. For legumes and other species with physical dormancy a determination of the proportion of hard diaspores is proposed as an alternative method to assess endozoochorous dispersal potential.

Introduction

Until today, endozoochorous dispersal of herbaceous species is treated very differently in dispersal ecology. This concerns the relevance of this mode of dispersal in general as well as its relevance for the dynamics of plant populations in the man-made landscape.

On the one hand, endozoochory of non-fleshy diaspores* often remained disregarded in the classical classification systems (e.g. LUFTENSTEINER 1982; VAN DER PIJL 1982; GRIME et al. 1988) or depreciated as "accidental endozoochory" (VAN DER PIJL 1982), where the diaspores are now and then ingested accidentally together with the proper feed. On the other hand, many studies have documented either by feeding experiments or by the collection of dung excreted by grazing animals that many plant species are capable to survive the passage of the digestive tract of ruminants (e.g. KEMPSKI 1906; GARDENER et al. 1993a; MALO & SUÁREZ 1995; STENDER et al. 1997; PAKEMAN et al. 2002). The main reason for the biased treatment of endozoochory is the lack of obvious morphological adaptations for endozoochorous dispersal by herbi- or omnivores (see also PAKEMAN et al. 2002), on which classification systems in dispersal ecology were based over decades (BONN & POSCHLOD 1998a; BONN et al. 2000; TACKENBERG et al. 2003).

In contrast to the obvious necessity of plant migrations and corresponding dispersal vectors after the last ice age (e.g. CAIN et al. 1998, 2000; CLARK et al. 1998; PAKEMAN 2001), the relevance of zoochorous dispersal by domestic livestock in cultural landscapes is only rarely emphasised for the maintenance of plant biodiversity (e.g. GARDENER et al. 1993a; MALO & SUÁREZ 1995; FISCHER et al. 1996; BONN & POSCHLOD 1998a,b; POSCHLOD & BONN 1998; chap. 2). However, today most of the Central European plant communities must be regarded as man-made or at least as modified by the various agricultural activities of man (ELLENBERG 1996). In this context, domestic livestock played especially in the traditional man-made landscape an outstanding role (ELLENBERG 1996; POSCHLOD et al. 1996a, 1997; BONN & POSCHLOD 1998a,b; POSCHLOD & WALLIS DEVRIES 2002). From the beginning of agriculture in the Neolithic ages on, the use of arable fields, forests and grasslands was connected inseparable with livestock breeding (BURRICHTER et al. 1993). On the one hand, deprived nutrients on arable fields had to be compensated by a subsequent fallow lasting for one or several years and the dung of grazing livestock (ABEL 1978). On the other hand, the lack of defined pastures resulted in a continuous rotation of domestic livestock to grazing areas, where feed supply could be covered and crop cultivation was not disturbed. The grazing of fallows included not only the regular field-rotations near the villages, but also the various shifting cultivations in forests, heath- or peatlands. In these farming systems the remnants of the used woods, the heath- or peatlands, were burnt for fertilization and cultivated for one or several years. After crop cultivation, the fields were grazed for several years and afterwards completely abandoned until the next rotation (e.g. ABEL 1978; SELTER 1995). Another important pasturage were the various commons, like heath- and wetlands in outlying areas of the village (RÖSENER

* The term »diaspore« is used for the unit of dispersal, which can be either a seed, various types of fruits or whole parts of the plant.

1986; FLAD 1987). Altogether, pasturages were represented over centuries by a big variety of habitats in space and time, which were connected by domestic livestock as dispersal vectors.

Against this background it becomes obvious that a consideration of the endozoochorous dispersal potential of plant species is crucial in the context of conservation biology in cultural landscapes in order to understand changes of plant communities and to predict plant biodiversity in a rapidly changing cultural landscape. Until today, however, a quantification of endozoochorous dispersal potential of plant species is not possible without conducting large-scale feeding experiments. Although correlations between various diaspore attributes and survival rates after gut passage of herbivores were found in different studies (e.g. size (SIMAO NETO et al. 1987; RUSSI et al. 1992), mass (THOMSON et al. 1990), specific gravity (GARDENER et al. 1993a)), the reliability of these correlates is still insufficient to predict endozoochorous dispersal potential for individual plant species (see also chap. 5). As an alternative to elaborate feeding experiments or an assessment of endozoochorous dispersal potential by morphological diaspore attributes, various methods simulating in vivo-digestion by in sacco- or in vitro methods have been attempted with more or less satisfying results (e.g. GOTO & MINSON 1977; JANZEN et al. 1985; SIMAO NETO & JONES 1987; OMED et al. 1989; OCUMPAUGH & SWAKON 1993; STERN et al. 1997; GODÍNEZ-ALVAREZ & VALIENTE-BANUET 1998; REMEŠOVÁ 2000). However, most of these methods were conducted only with grasses and legumes, included either a digestion step with rumen liquid from fistulated animal, which seems undesirable as a standard method for large-scale experiments, or gave unsatisfying correlations with survival rates after in vivo-digestion.

Despite the deficits of existing approaches trying to simulate digestion, an experimental approach seems to be the most exact way to quantify endozoochorous dispersal potential of individual plant species. However, the relevance of single steps of the ruminant digestive system for diaspore survival is not yet clear. A diaspore may die during digestion because it is damaged by the grinding action of the teeth or the microbial and chemical processes in the alimentary tract, or by simple imbibition in the anoxic digestive juices. The unsatisfying correspondence of survival rates after in vivo-digestion and different in vitro procedures (SIMAO NETO & JONES 1987), for example, may result from the missing consideration of mechanical feed comminution during mastication in all existing simulation methods. Chewing and remastication during rumination play a major role for particle breakage, whereas microbial comminution in the rumen is evaluated as less important (e.g. MCLEOD & MINSON 1988; LUGINBUHL et al. 1989; WILSON et al. 1989). Furthermore, a mechanical fractioning of particles is an important precondition for a further digestion of feed by ruminal microbes (MCALLISTER & CHENG 1996). Including a mechanical digestion step when simulating the ruminant digestive system might therefore be helpful to improve correlations between in vivo-survival rates of diaspores and experimental approaches simulating digestion.

Consequently, it was intended by the present study:

- to identify the effect of single steps of the ruminant digestive system on diaspore viability;
- to identify the role of certain components of the digestion juices (e.g. enzymes) for the survival of ingested diaspores;

- to develop an adequate and preferably simple method to classify species with regard to their dispersal potential via the digestive tract of ruminants

using different experiments simulating mastication, ruminal and postruminal digestion.

Methods

Feeding experiment

The feeding experiment was conducted at the IGER Institute in Okehampton, England, with five one year old limousine heifers (mean body weight 300 kg) and five male two year old Texel sheep (mean body weight 60 kg). The animals were fed with a silage of 70% digestibility. In order to guarantee a complete uptake of the diaspores together with the feed, the animals were adapted to bruised grain three days before the beginning of the feeding experiment.

Table 4.1 Plant species, quantities and viability of diaspores fed to cattle and sheep.

species	abbre-viation	family	diaspore origin	viability before feeding	viable dia-spores fed per animal
Achillea millefolium L.	Ach mil	Asteraceae	wild form	95.9%	5754
Anthyllis vulneraria L.	Ant vul	Fabaceae	wild form	76.8% (37.8% hard seeds)	4608
Festuca ovina *agg.*	Fes ovi	Poaceae	cultivated form	38.2%	2292
Hieracium pilosella L.	Hie pil	Asteraceae	wild form	65.4%	3924
Inula conyza DC.	Inu con	Asteraceae	wild form	24.6%	1476
Jasione montana L.	Jas mon	Campanulaceae	wild form	36.9%	2214
Leucanthemum vulgare LAMK.	Leu vul	Asteraceae	cultivated form	81.4%	4884
Lotus corniculatus L.	Lot cor	Fabaceae	cultivated form	87.2% (0.6% hard seeds)	5232
Pimpinella saxifraga L.	Pim sax	Apiaceae	cultivated form	71.4%	4284
Plantago media L.	Pla med	Plantaginaceae	wild form	64.2%	3852
Poa angustifolia L.	Poa ang	Poaceae	wild form	60.0%	3600
Salvia pratensis L.	Sal pra	Lamiaceae	wild form	61.4%	3684
Sanguisorba minor SCOP.	San min	Rosaceae	wild form	94.5% (1.6 embroys/ diaspore)	8900
Thymus pulegioides L.	Thy pul	Lamiaceae	wild form	75.9%	4554

The experiment included 14 plant species of eight plant families (Table 4.1). Species were selected according to the following criteria:

- occurrence on calcareous grasslands (which often have developed as a result of livestock-grazing);
- belonging to different plant families;
- different diaspore attributes (diaspore size, morphology, weight, occurrence of hard seeds);
- availability of a large number of diaspores.

6,000 diaspores (commercial seed) of each of the 14 plant species were mixed into bruised grain and fed to each animal. In total, 60,000 diaspores of each plant species were fed. Sheep were separated in single pens during the experiment. The pens were covered with paper shavings, which simplified dung collection after diaspore feeding. Because cattle could not be separated, colored plastic markers were fed together with the diaspores as well as one day before and two days after diaspore feeding together with the regular feed. The dung of each individual could then be distinguished by the different colored plastic markers in the excreted faeces. The cattle stable was covered with straw, where a quantitative dung collection proved to be more difficult then in the case of the sheep.

Dung collection started 12h after feeding the diaspores to sheep and 17h after diaspore feeding to cattle. Faecal material was collected three times per day over a period of eight days (166h) in the case of sheep and nine days (191h) in the case of cattle, because of different retention times in sheep and cattle (e.g. LENNARTZ 1955; RUSSI et al. 1992; overview in BONN & POSCHLOD 1998a). During the last three collection days, the dung of only one sheep or cattle was collected, since it was expected that most diaspores were already excreted (e.g. RUSSI et al. 1992; GARDENER et al. 1993a). Each dung sample was weighed and subsequently dried at room-temperature in order to promote germination (GRIME et al. 1988). 25% of each dung-sample were crumbled, rewetted, mixed with sterile compost and filled in a 1 cm thick layer in pots, separated after day and time of collection. Faecal material of the last three collection days was exposed to 100%. The samples were exposed in random order in the botanical garden at the University of Marburg (Germany) under natural weather conditions, but wetted if necessary. In order to avoid diaspore input from outside, all pots were covered by a garden fleece. Seedlings were identified and removed in regular time intervals over a period of 15 months, which included a stratification period during winter.

Initial viability of the fed diaspores was determined by germination experiments in a climate chamber. 100 to 150 diaspores of each plant species (5 replications) were placed on two filters in petri dishes. Petri dishes were sealed with parafilm and exposed under a 14h light/22°C, 10h dark/14°C climate-regime. Germinated diaspores were counted and removed twice per week until germination had finished. Ungerminated diaspores were tested for remaining hard (legumes) or firm (grasses, forbs) ungerminated diaspores (ROBERTS 1981; DOUCETTE et al. 2001). Both germinated and hard or firm ungerminated diaspores were summed up to calculate the viability of a species.

Undigested diaspores, exposed under identical conditions as the digested diaspores, were used as a control. Therefore, 100 diaspores of each plant species were brought out on cattle faeces mixed with sterilised compost. Cattle dung was collected immediately after voiding and preserved in closed plastic bags at 4°C until the exposure in the botanical garden. These controls were exposed for the same time period as the dung samples from the feeding experiment in the botanical garden. The germination success of fed diaspores in the exposed dung samples was then related to germination of undigested diaspores exposed on dung ("relative germination"). Since it was supposed that all viable diaspores germinated during dung exposure, relative germination of fed diaspores was equated with diaspore survival.

Approaches to predict the capacity of diaspores to survive the passage through the ruminant digestive system

In former studies mostly two-staged approaches were used to assess the digestibility of animal feed or the survival capacity of diaspores (e.g. TILLEY & TERRY 1963; SIMAO NETO & JONES 1987; OCUMPAUGH & SWAKON 1993). They simulate microbial digestion in the reticulo-rumen and subsequent acid digestion in the abomasum and small intestine. However, several studies show that chewing plays a major role for particle size reduction (LEE & PEARCE 1984; LUGINBUHL et al. 1989; WILSON et al. 1989). In the present study, ruminant digestion was therefore divided into three superior digestion steps, which may be critical for the survival of diaspores during gut passage (Fig. 4.1, Table 4.2).

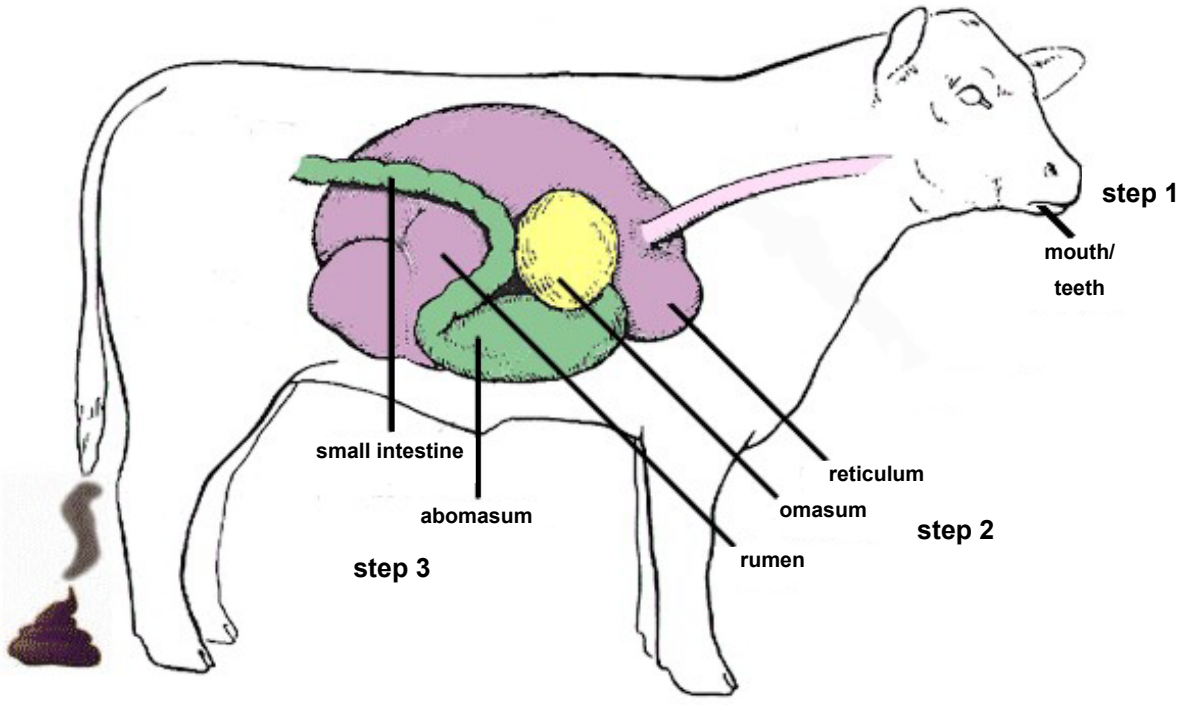


Fig. 4.1 The ruminant digestive system with its digestion steps relevant for diaspore survival.

Table 4.2 Major digestion steps of the ruminant digestive system, the effects on ingested feed and hypothesized effects on ingested diaspores (HOWARD 1986; MCALLISTER & CHENG 1996; GORDINEZ-ALVAREZ & VALIENTE-BANUET 1998).

digestion step	effects on ingested feed	hypothesized effects on a diaspore
1. Teeth/ mastication and rumination	singular to repeated mechanical comminution of feed	seed coat may be mechanically scarified; mechanical damage of the embryo
2. Fore-stomach with reticulum, rumen and omasus	digestion in an anoxic liquid with cellulolytic enzymes lasting for several hours to several days ("microbial digestion")	seed coat may be chemically (enzymatically) scarified; the diaspore may imbibe and the seed or fruit coat may rupture in the anoxic fluid; chemical damage of the embryo
3. Abomasus and small intestine	digestion in a strongly acid milieu with proteolytic enzymes (pepsin)	seed coat may be chemically scarified by HCl, the diaspore may imbibe or even germinate in a strongly acid milieu; chemical damage of the embryo

Table 4.3 Experiments (rows) to simulate different digestion steps of ruminants (columns) relevant for diaspore survival during passage of the digestive tract.

Grey cells indicate the digestion steps simulated by an experiment.

experiment	step 1 mastication/ rumination	step 2 ruminal digestion	step 3 postruminal digestion
I. standardized mechanical stress			
II. standardized mechanical stress + immersion in hydrochloric acid			HCl
III. in vitro-digestion with rumen fluid and HCl-pepsin (hours to days)		anoxic liquid + enzymes	HCl-pepsin
IV. imbibition in anoxic liquid (water) (hours to days) + immersion in hydrochloric acid		anoxic liquid	HCl
V. immersion in hydrochloric acid			HCl

For the experimental approaches (Table 4.3) all species of the feeding experiment were used except of *Jasione montana* and *Thymus pulegioides*. Diaspores of *Jasione* proved to be too small for a quantitative handling, whereas those of *Thymus* were used up during the feeding experiment and were no longer available. If not described else, the experiments were conducted with 5 replicates, each with 100 to 150 diaspores per species. For each experiment, germination success after a treatment as well as germination of untreated diaspores (control) were analyzed in petri dishes provided with two wet filter papers and exposed under a 14h light/22°C, 10h dark/14°C

climate-regime until germination had finished. Remaining diaspores were tested for hardseededness (legumes) and firmness (grasses, forbs) to determine total viability (ROBERTS 1981).

Diaspore viability after the different methods simulating single steps of digestion was related to diaspore viability of untreated diaspores ("relative survival").

Simulation of mastication and chemical attacks in the abomasum (Table 4.3: I, II and V)

Fig. 4.2 Simulation of chewing.

Table 4.4 Factors and intensities used for simulation of chewing and subsequent immersion in hydrochloric acid.

factors	intensities
1) chewing	3 (no chewing/ 2x / 4x)
2) acid treatment (0.1 M HCl)	3 (no acid treatment / 2h / 8h)

Feed comminution by chewing is not only the result of a force acting on feed particles, but also results from shear strength (e.g. KENNEDY & DOYLE 1993). These shear forces are exercised by a rotary or horizontal movement of the lower jaw to grind fibres (PÉREZ-BARBERÍRA & GORDON 1998; BUNGO et al. 1999). The frequency of chewing feed is difficult to quantify, because it depends on individual as well as on nutritional parameters (LEE & PEARCE 1984; KENNEDY & DOYLE 1993; MTENGETI et al. 1995). Furthermore, efficiency of chewing on particle breakage depends on the structure of feed (MTENGETI et al. 1995).

The following standardized method was chosen to assess diaspore sensitivity to mechanical stress: an iron stick with a contact area of 2 cm² and a length of 1.3 m was burdened with body weight (~70 kg) and moved 90° lateral (= 1 chew, Fig. 4.2). The top of this iron "chewing stick" was surrounded by one layer of fleece material and two layers of rubber tape in order to simulate the contact of diaspores with the teeth buffered by other feed material. The chewing area of the "chewing stick" was fitting exactly into plastic lids, which were affixed to a wooden board "representing" the lower jaw. Diaspores were filled into the lids in a single layer covering completely the area of the lid and "chewed" either two or four times (2 or 4 rotations at 90° each) (Table 4.4).

If a seed coat is slightly damaged, the diaspore may still germinate although it will be much more susceptible to chemical attacks occurring in the digestive tract. Therefore, "chewed" and – for comparison – untreated diaspores were immersed in 0.1 M HCl simulating the acid environment in the abomasum and small intestine without enzymes. Diaspores were put into small glass tubes filled with HCl for two or eight hours (Table 4.4) and afterwards washed with H_2O dest. on a porcelain filter.

Simulation of digestion in the reticulo-rumen and abomasum by in vitro-digestion (Table 4.3: III)

The in vitro-digestion experiment was conducted at the institute for Animal Nutrition in the Tropics and Subtropics, workgroup Rumen Ecology, at the University of Hohenheim. The incubation medium for the simulation of ruminal digestion was based on the Hohenheim Gas Test (MENKE et al. 1979; VDLUFA 1997). Because of varying retention times in the reticulo-rumen (e.g. KASKE & ENGELHARDT 1990), but relative constant retention times in the abomasum and lower gastrointestinal of maximal 10h (CHURCH 1988), the two-stage digestion procedure consisted of a series of incubation times in a first digestion step with rumen liquid followed by an 8h digestion in acidified pepsin. Because survival rates were expected to decrease with increasing incubation time, the number of incubated diaspores was doubled from 24h simulated rumen digestion on in order to get a sufficient number of viable diaspores. Diaspores of each species were put into small nylon bags (aperture size 130 µm), closed with a nylon thread. When doubling the number of diaspores from 24h rumen digestion on, the diaspores were put into two separate nylon bags. The nylon bags of all species per incubation time and replicate were put together into coarse meshed nylon nets (aperture size ~0.7 cm).

All samples of one replicate (n=5) were put together into one bottle for simulated ruminal digestion over six different time intervals (6h, 12h, 24h, 48h, 72h, 120h), each followed by an acid pepsin-digestion over 8h. Shortest and longest total incubation time were conformed to observed retention times of in vivo-digestion.

In vitro-digestion - stage I

Rumen liquid was taken from three cannulated cows (two Holstein cows and one Simmentaler[*]), fed with a hay and silage diet of intermediate feed quality supplemented by small amounts of concentrated feed. Rumen liquid was taken twice on two following days from the two Holstein cows and once from the Simmentaler cow. Animal and day of rumen liquid extraction were taken as replicates (n=5). After extraction, rumen liquid was filtered under CO_2-gassing through a 100 µm net until a volume of 1.5 liters of pure rumen liquid was obtained.

Each replicate with a total volume of 4.5 liters, consisting of fresh collected, filtered rumen liquid, minerals, buffer and H_2O bidest (Table 4.5, MENKE et al. 1979; VDLUFA 1997), was incubated in a 5 l bottle. Since diaspores were not expected to sufficiently substitute the feed normally digested, microbial activity might have been suppressed without adding additional feed (s. also OCUMPAUGH & SWAKON 1993). Therefore, milled hay (≤ 1 mm) of intermediate feed quality which was also fed to the cows was added to the incubation medium (Table 4.5). Bottles were preserved in a climate chamber at 39°C and shaken occasionally to reduce a separation of the liquid into two phases.

[*] As shown by OCUMPAUGH & SWAKON (1993), the source of rumen fluid has no effect on survival rates of exposed diaspores, provided a similar feeding of the donor animals.

In order to avoid an inhibition of ruminal microbes by end products from the digestion, 60% of the incubation medium were exhausted under CO_2-gassing every 24h and replaced by the same volume with fresh Hohenheim Gas Test medium as well as with milled hay (the original amount of 5 mg/ml incubation medium was reduced by 10% at each exchange).

In vitro-digestion - stage II

To simulate digestion in the abomasum and small intestine, an acidified pepsin solution (0.1 M HCl; 2 g pepsin/l) according the first step of the cellulase method (VDLUFA 1993, based on DE BOEVER et al. 1986) was used. Each nylon net with a complete species set was put for 8h under constant motion into 1l acidified pepsin solution at 39°C.

After the treatment, the diaspores were washed under running water and tested for viability as described above.

Table 4.5 Composition of the solution for in vitro-simulation of ruminal digestion based on the Hohenheim Gas Test (MENKE et al. 1979; VDLUFA 1997).

liquid/compartment	amount
rumen liquid	1500 ml
buffer solution	714 ml
macro-minerals	714 ml
micro-minerals	0.714 ml
Resazurine	0.893 ml
H_2O dest	1569 ml
milled hay (particle size ≤ 1 mm)	7 mg/ml incubation medium
sodium sulfite	893 mg
NaOH	0.952 ml

Diaspore incubation in anoxic liquid and subsequent immersion in HCl (Table 4.3: IV and V)

Although microbial digestion in the rumen is known to play a major role in feed digestion, viability of ingested diaspores may already be reduced by the mere stay in the warm and moist but anoxic environment of the rumen over several hours or days, because processes like imbibition or even germination may be initialized. Even if a diaspore survives the stay in the anoxic rumen juices, it may be more susceptible in the subsequent acid environment of the abomasum and small intestine.

In order to test this, diaspores of all species* were incubated in H_2O dest. and continuously bubbled with nitrogen. The oxygen content of the water amounted to ~0.09 mg/l, which is

* *Sanguisorba minor* and *Lotus corniculatus* were not available from the seed lots used for all other experiments. Therefore, diaspores from other origins had to be used.

comparable to the oxygen content of rumen liquid (SCOTT et al. 1983; HILLMAN et al. 1985).

Diaspores (2 g) of each species were put into small nylon bags (aperture size 130 µm). The nylon bags of all twelve species were put together into coarse meshed nylon nets (aperture size 0.7 cm). The complete species sets were incubated over six different time intervals (6h, 12h, 24h, 48h, 72h and 120h) with 5 replicates into the anoxic water. Each of the five replicates was put into a single water-filled bottle bubbled with nitrogen. After the incubation in anoxic water the diaspores were immersed in 0.1 M HCl for 8 hours.

In order to compare these treatments with the mere effect of the acid environment of the abomasum and small intestine, one treatment only included an immersion into 0.1 M HCl for 8 hours.

After each treatment, the diaspores were washed and tested for viability as described above.

Data analysis

The results of the feeding experiment were analyzed with regard to percentage of recovery in relation to diaspore viability determined in the climate chamber and the botanical garden (relative germination or survival, respectively) and time dependent excretion rate. Time of 50% diaspore excretion was calculated by interpolating between the single collection dates. Since data of diaspore survival showed no normal distribution, relative germination was compared between species as well as between ruminants using a non-parametric Kruskal-Wallis H-Test. Pair-wise comparisons were assessed by Mann-Whitney U-test. The same tests were used to analyze differences in the time, when 50% of all recovered diaspores were excreted. Analyses were performed to compare 50% recovery rate among different plant species fed to a certain animal as well as between cattle and sheep.

Relative survival resulting from simulated chewing was square-root transformed to meet requirements for analysis of variance (normality and homoscedasticity). Homoscedasticity was tested using the F_{max}-test. Analyses were performed with absolute as well as with relative survival. Absolute survival of each species after different intensities of acid and mechanical treatment was compared by one-way ANOVA, followed by pair-wise Scheffé-tests. In order to identify interactions of mechanical- and acid-treatment, a two-factor ANOVA was performed.

Differences in relative survival after in vitro-digestion among species were tested using a Kruskal-Wallis H-Test, pair-wise comparisons were made with Mann-Whitney U-tests. In order to compare relative survival of diaspores after in vivo- and in vitro-digestion, the average of diaspore survival of all six incubation times of the in vitro-digestion was calculated. The same analysis was done for diaspores incubated in anoxic liquid, followed by an incubation in 0.1 M HCl.

Comparisons of survival after the different experimental treatments with survival after passing the digestive tract of cattle or sheep were performed using Pearson's correlation coefficients after arcsine square-root transformation of all data.

Statistical analysis was done using SPSS (Vers. 11.0).

Results

Survival of plant species fed to cattle and sheep

Mean numbers of diaspores germinating in the dung of sheep and cattle were statistically similar (2,126 ± SE 172.1 and 2,153 ± SE 183.0 seedlings, respectively). 3.6% of all viable diaspores fed germinated in the dung of both animal species. However, also the germination of undigested diaspores exposed on the dung-soil mixture in the botanical garden was for all species lower than in the climate chamber (Fig. 4.3). Except for *Festuca ovina* and *Sanguisorba minor*, this difference was for all species significant ($p<0.01$, U-Test). Viability in the climate chamber was correlated with germination rates of undigested diaspores on dung ($r=0.71$, $p<0.01$, Pearson's correlation coefficient).

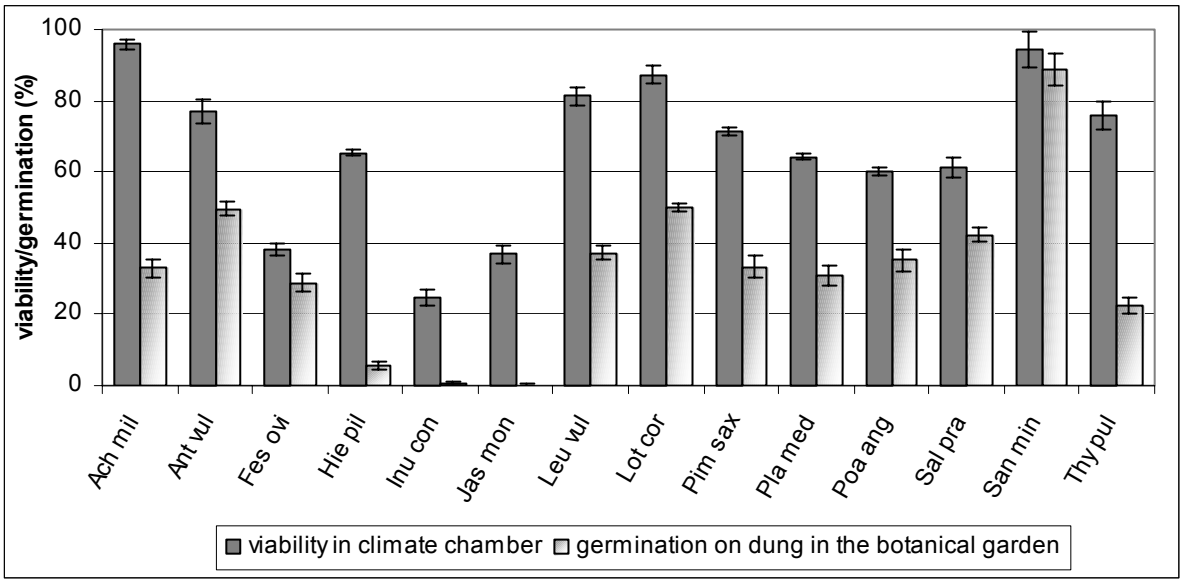


Fig. 4.3 Diaspore viability in the climate chamber in relation to the germination of untreated diaspores exposed on a dung-soil mixture in the botanical garden.

Bars: ± SE; abbreviations for plant species see Table 4.1.

Despite similar mean numbers of germinated seedlings in sheep and cattle dung, there was a big variance between individual animals and among plant species. Regarding the fed plant species, significant differences in diaspore survival after passing the gut of cattle and sheep were found for five species (Table 4.6): *Plantago media, Thymus pulegioides* ($p<0.01$), *Hieracium pilosella* and *Salvia pratensis* ($p<0.05$) survived the passage of the sheep gut better, whereas *Sanguisorba minor* survived the passage of cattle's digestive tract in higher rates ($p<0.01$). *Jasione montana* and *Inula conyza* germinated neither in sheep nor in cattle dung after passing the digestive tract. However, both species even showed extremely low germination rates when undigested diaspores were sown on dung (Fig. 4.3). Therefore, an accurate assessment of species

survival after a further reduction of diaspore viability by the ruminant digestive system was nearly impossible because seedling numbers would have been too low.

Diaspore feeding to sheep resulted in similar seedling numbers of *Anthyllis vulneraria* and *Achillea millefolium* in the excreted dung (Table 4.6). Both species survived gut passage in significantly higher quantities ($p<0.01$) than all other species. With a relative survival between 1.8 and 1.7%, *Sanguisorba minor* and *Hieracium pilosella* differed from all species with a higher survival but *Festuca ovina* ($p<0.05$). Of those species, where any diaspore germinated in sheep dung, *Salvia pratensis* and *Pimpinella saxifraga* showed the lowest survival rates.

Anthyllis vulneraria and *Achillea millefolium* also obtained highest seedling numbers in the dung excreted by cattle (Table 4.6). Again, diaspores of *Pimpinella saxifraga* and *Salvia pratensis* germinated in very low quantities. In contrast to sheep dung, seedlings of *Sanguisorba minor* were found in relatively high quantities in the exposed cattle dung.

Table 4.6 Mean relative survival of 14 plant species fed to cattle and sheep (± SE).

Tests for differences in relative survival between sheep and cattle were done separately for each plant species by U-Test; ** = $p<0.01$; * = $p<0.05$;
tests for significant differences among plant species fed to sheep or cattle by H-Test, different letters indicate differences in survival among species fed either to cattle or sheep at $p<0.05$ by U-Test.

	Relative survival (%) of diaspores fed to …		
	sheep	**cattle**	
Anthyllis vulneraria	26.1 ± 2.9 ^{a}	34.2 ± 2.3 ^{a}	
Achillea millefolium	24.8 ± 2.5 ^{a}	16.3 ± 1.9 ^{b}	
Plantago media	9.8 ± 2.0 ^{b}	4.9 ± 0.3 ^{cde}	**
Poa angustifolia	8.6 ± 2.7 ^{bcd}	5.3 ± 0.6 ^{cd}	
Thymus pulegioides	7.2 ± 1.3 ^{bc}	1.5 ± 0.2 ^{l}	**
Leucanthemum vulgare	6.0 ± 1.2 ^{bcd}	4.3 ± 0.4 ^{def}	
Lotus corniculatus	4.4 ± 0.3 ^{d}	3.4 ± 0.5 ^{efg}	
Festuca ovina	2.6 ± 0.9 ^{de}	1.3 ± 0.2 ^{ij}	
Sanguisorba minor	1.8 ± 0.6 ^{ef}	6.9 ± 0.8 ^{c}	**
Hieracium pilosella	1.7 ± 0.3 ^{ef}	3.0 ± 0.3 ^{fgh}	*
Salvia pratensis	0.3 ± 0.1 ^{g}	0.1 ± 0.0 ^{kl}	*
Pimpinella saxifraga	0.2 ± 0.1 ^{g}	0.3 ± 0.1 ^{k}	
Inula conyza	0.0 ± 0.0 ^{h}	0.0 ± 0.0 ^{l}	
Jasione montana	0.0 ± 0.0 ^{h}	0.0 ± 0.0 ^{l}	

Diaspore excretion as a function of time

Time span of diaspore excretion varied greatly among individual animals. This accounts especially for the excretion of *Poa angustifolia*-diaspores by individual sheep or *Festuca ovina* and most plant species with very low survival rates after passing the alimentary tract of both animal species.

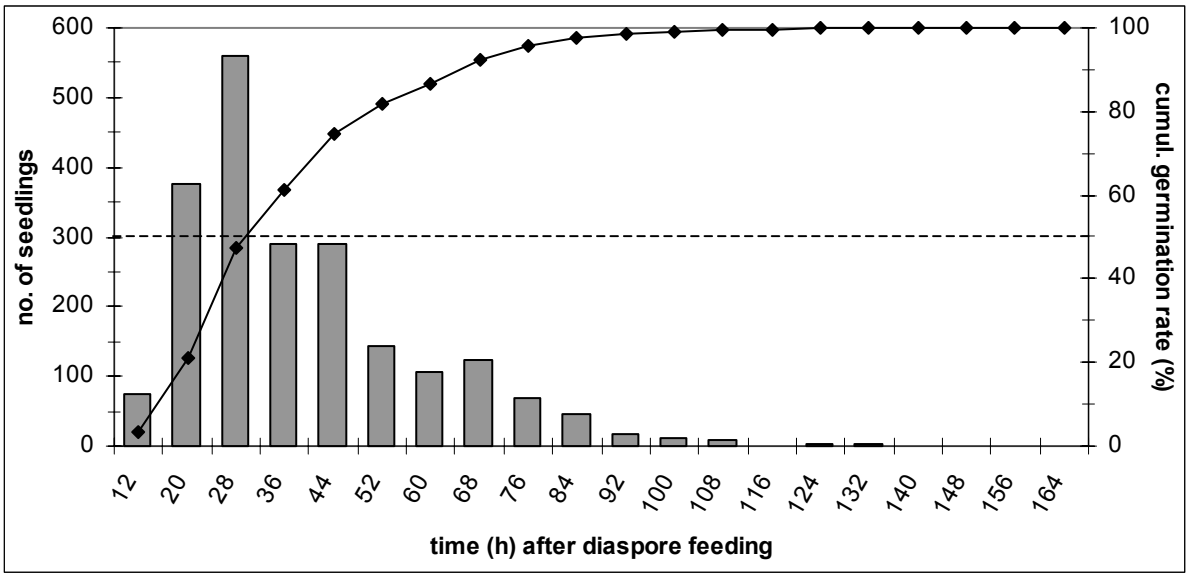


Fig. 4.4 Number of seedlings of all plant species germinating in the excreted sheep dung taken at different time steps after feeding.

Dotted line: 50% excretion rate.

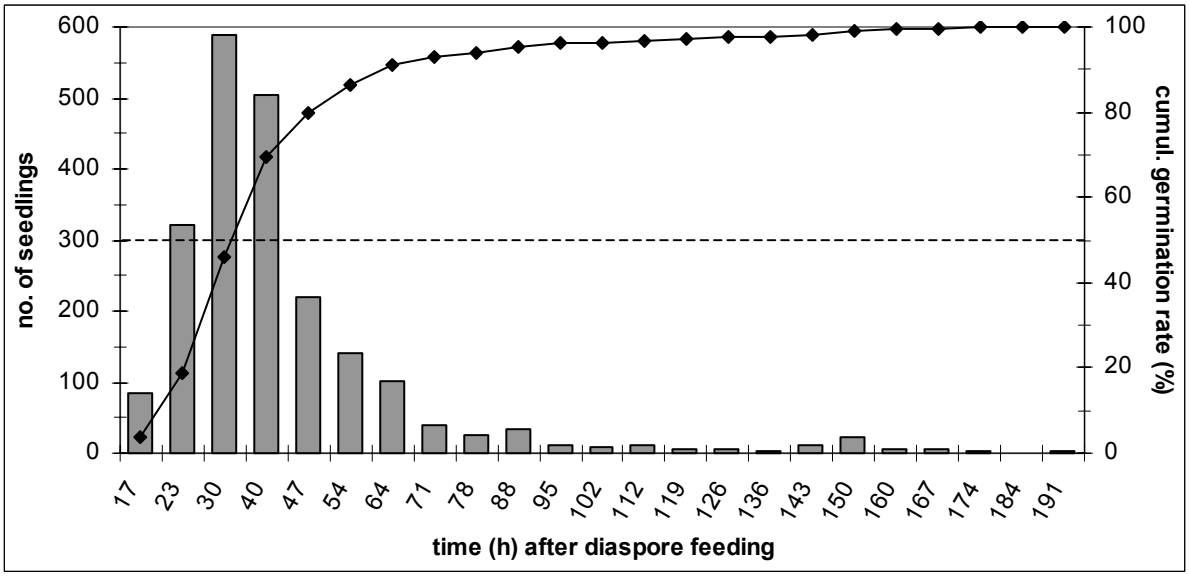


Fig. 4.5 Number of seedlings of all plant species germinating in the excreted cattle dung taken at different time steps after feeding.

Dotted line: 50% excretion rate.

Already 12h after diaspore feeding, germinable diaspores of all fed species were present in the faeces of sheep (Fig. 4.4). Maximum numbers were recovered between 20h and 28h after diaspore feeding except for *Hieracium pilosella* (maximum after 36h) which, however, showed in general a very low survival rate. Four days after feeding, diaspores were recovered only irregularly and in few numbers. 30h post feeding, 50% of all diaspores were excreted. *Anthyllis vulneraria* was continuously excreted up to 124h after diaspore feeding and passed the digestive tract of sheep slower than most other species (Table 4.7).

As in sheep, some diaspores of most species fed had already passed the digestive tract of cattle at the beginning of dung collection 17h after diaspore feeding. Maximum recovery rates could be observed some hours later than in sheep dung (Fig. 4.5). During the second day after diaspore feeding the highest numbers of germinable diaspores were excreted (30h to 40h). 32h post feeding, 50% of all diaspores had passed the digestive tract of cattle. According to sheep, both legumes as well as *Festuca ovina* belonged to those species with the longest time until 50% of all recovered diaspores were excreted (Table 4.7). However, for single species (e.g. *Thymus pulegioides*) there could be observed also great differences in the mean time for 50% diaspore excretion between the two animal species.

Table 4.7 Mean times for 50% diaspore excretion when passing the digestive tract of sheep and cattle. Significant differences among species by H-Test; different letters indicate significant differences in excretion rate among species at $p<0.05$ by U-Test.

sheep	mean time (h) for 50% diaspore excretion	SE	cattle	mean time (h) for 50% diaspore excretion	SE
Festuca ovina	39.0 ab	12.6	*Festuca ovina*	49.8 abcde	15.6
Anthyllis vulneraria	39.0 a	4.3	*Thymus pulegioides*	49.5 abde	15.9
Poa angustifolia	37.8 abc	9.5	*Lotus corniculatus*	39.3 b	2.3
Lotus corniculatus	37.0 ad	5.0	*Anthyllis vulneraria*	38.0 bc	1.7
Hieracium pilosella	31.6 abc	6.9	*Pimpinella saxifraga*	31.8 abde	3.5
Achillea millefolium	26.2 b	0.8	*Leucanthemum vul.*	30.5 ad	1.7
Plantago media	25.1 bd	1.8	*Poa angustifolia*	30.1 d	1.5
Leucanthemum vul.	24.6 b	0.8	*Plantago media*	28.6 ade	1.7
Thymus pulegioides	23.9 b	3.3	*Achillea millefolium*	27.4 ade	1.1
Sanguisorba minor	23.9 b	2.0	*Salvia pratensis*	26.5 abde	0.0
Salvia pratensis	19.3 b	5.7	*Sanguisorba minor*	26.1 ae	1.0
Pimpinella saxifraga	14.5 c	2.7	*Hieracium pilosella*	20.4 e	3.7

Simulation of diaspore passage through the digestive tract of ruminants

Simulation of mastication and chemical attacks in the abomasum

Species sensitivities to different acid and mechanical treatments were very variable. A bath in 0.1 M HCl for 2h already reduced diaspore viability of two-thirds of all tested species (Fig. 4.6). Only *Poa angustifolia* showed no significant reduction of viability even after 8h in HCl. In contrast, viability of several species was reduced after 2h HCl-treatment, but an extension to 8h led to no further reduction of viable diaspores (*Anthyllis vulneraria, Festuca ovina, Leucanthemum vulgare and Plantago media*). Diaspores of *Inula conyza* were completely killed after an immersion of only 2h in HCl. Only for two species (*Lotus corniculatus* and *Pimpinella saxifraga*) a continuous reduction of viability with increasing time in HCl could be observed.

Reactions to mechanical stress were also variable among the tested species. Except of *Lotus corniculatus* and *Poa angustifolia,* all species showed a reduction of viability after only two simulated chews (Fig. 4.7). However, viability of most species was not further reduced when mechanical strain was extended from two to four simulated chews. Only *Leucanthemum vulgare* and *Pimpinella saxifraga* showed an increasing percentage of killed diaspores.

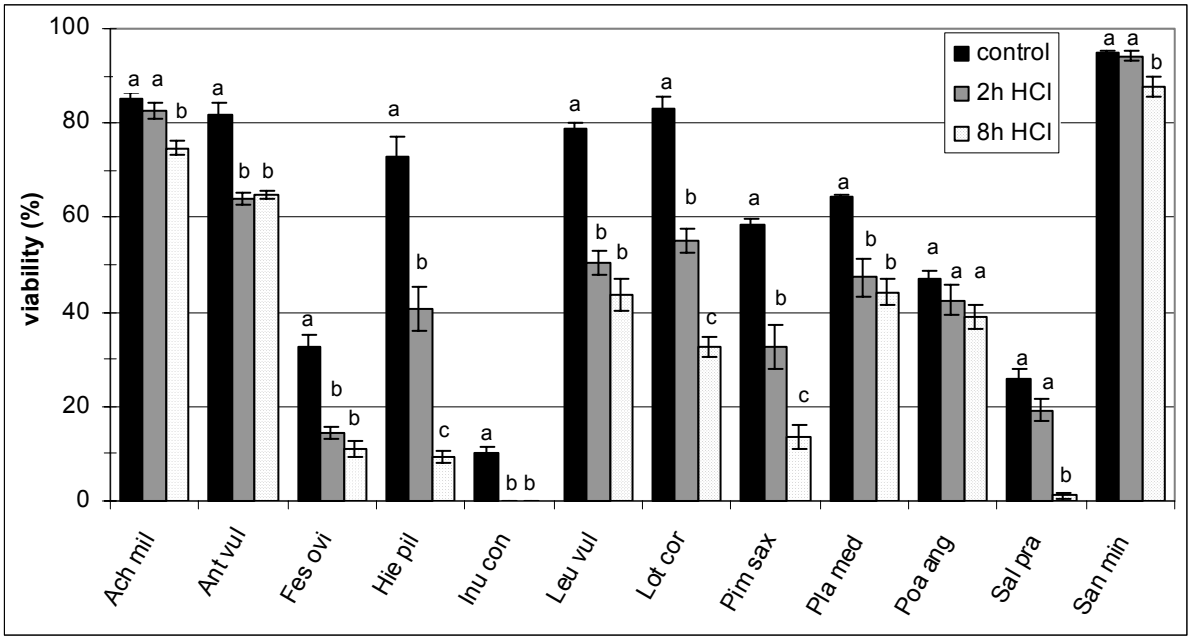


Fig. 4.6 Viability of species without acid treatment (control) and after an immersion in 0.1 M HCl for 2h or 8h.

Bars: ± SE; differences per species by one-factor ANOVA, Scheffé-test for pair-wise comparisons; different letters indicate significant differences between treatments at $p<0.05$, abbreviations for plant species see Table 4.1.

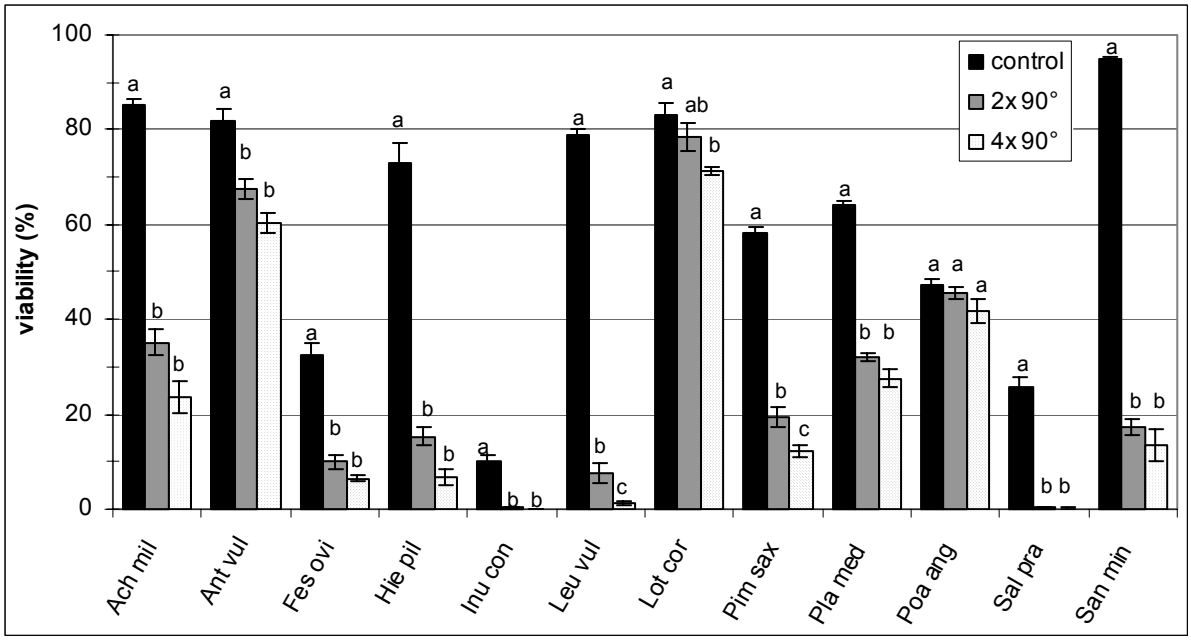


Fig. 4.7 Viability of species without simulated chewing (control) and after two or four simulated chews.

Bars: ± SE; differences per species by one-factor ANOVA, Scheffé-test for pair-wise comparisons; different letters indicate significant differences between treatments at $p<0.05$, abbreviations for plant species see Table 4.1.

Figure 4.8 shows the interaction between the mechanical and chemical diaspore treatment. A combination of mechanical and chemical stress led for all species to a distinct decline of survival rates. For half of the tested species two-factor ANOVA revealed significant interactions between mechanical and hydrochloride-acid treatment ($p<0.01$). Several relatively acid-tolerant species reacted often sensitive to mechanical stress (e.g. *Achillea millefolium, Leucanthemum vulgare, Sanguisorba minor*). At least at the less intense acidic treatment, *Hieracium pilosella, Pimpinella saxifraga* and *Salvia pratensis* proved also being more sensitive to the mechanical strain. In contrast, *Lotus corniculatus* lost more viable diaspores during both hydrochloride-acid treatments (2h and 8h) than by mechanical strain.

Survival after chemical and/or mechanical treatments showed no correlation with any morphological diaspore attribute. Neither diaspore length, width, height or shape nor different characteristics of the seed coat (total thickness, thickness of single layers or phenol content, see chap. 5) gave any significant correlation (Pearson's correlation, $p<0.05$).

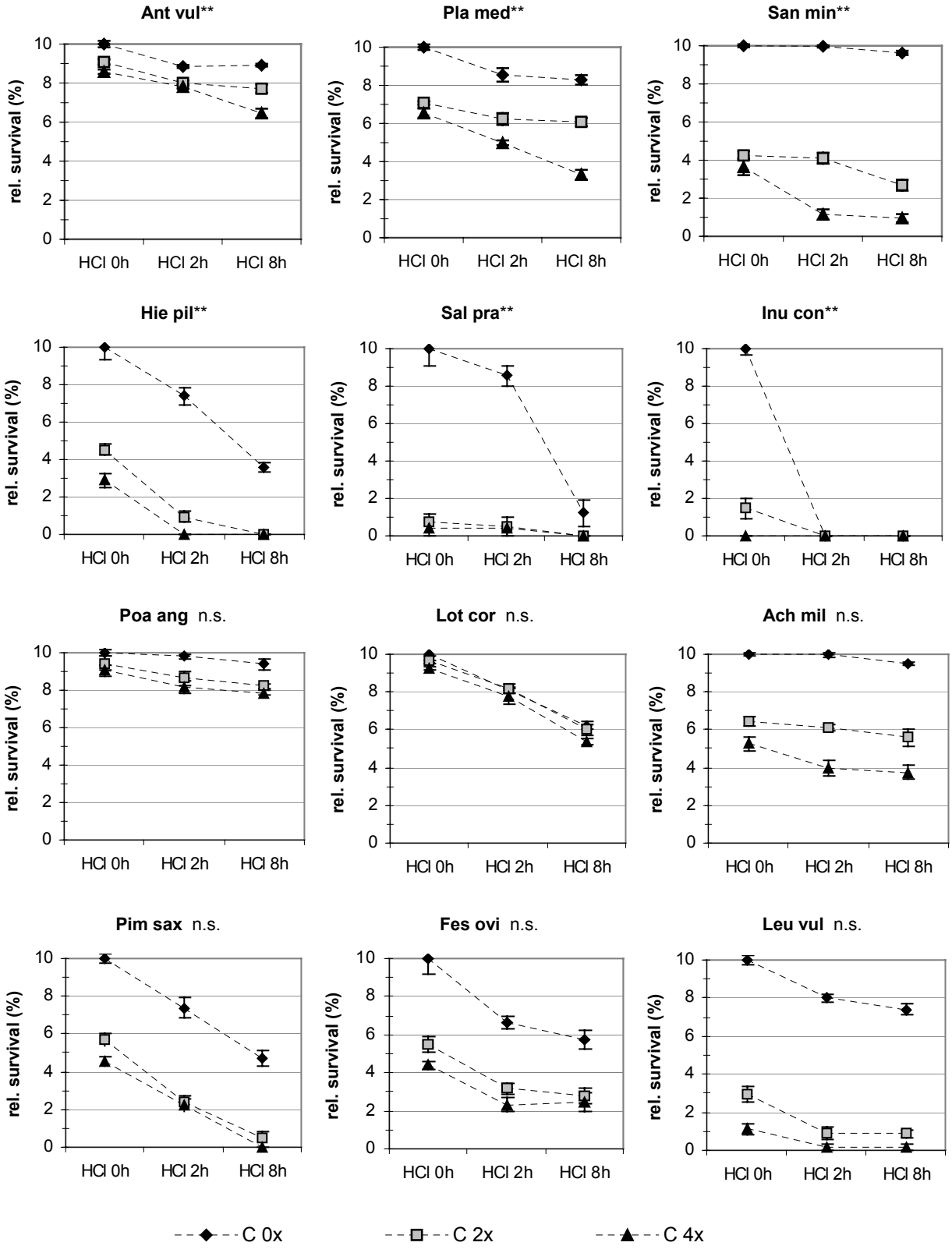


Fig. 4.8 Relative survival after continuing chemical (0h, 2h, 8h in 0.1 M HCl) and mechanical treatments (C 0x = without simulated chewing; C 2x = 2 simulated chews; C 4x = 4 simulated chews). Bars: ± SE; two-factor ANOVA proves for significant interactions of treatments, pair-wise comparisons were done by Scheffé-test: ** $p<0.01$; n.s.= not significant; all data are square-root transformed; abbreviations for plant species see Table 4.1.

Simulation of the digestion process in the reticulo-rumen and the abomasum by in vitro-digestion

Averaging diaspore survival of all six incubation times of in vitro-digestion (Fig. 4.9) illustrates that survival rates were for most species higher then those after in vivo-digestion (Table 4.6, Fig. 4.13). Contrary to the feeding experiment, *Poa angustifolia* survived the in vitro-digestion best (Fig. 4.9). *Salvia pratensis* and, in accordance to the results of the in vivo-digestion, *Inula conyza* did not survive even the shortest incubation time. A markedly lower survival than after in vivo-digestion could be observed for *Lotus corniculatus*.

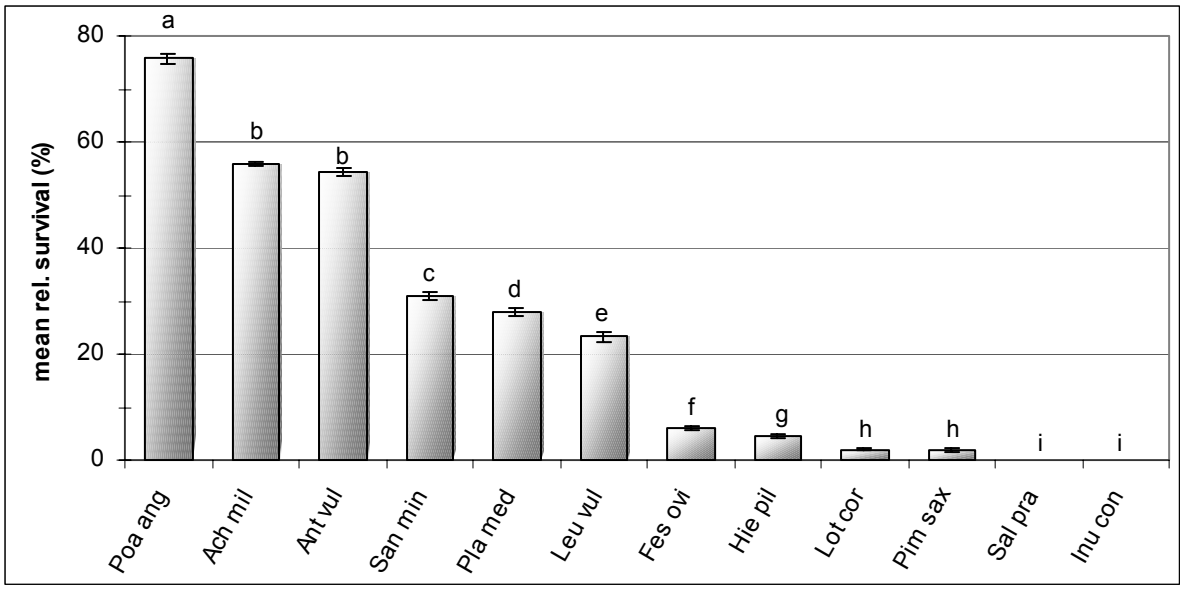


Fig. 4.9 Mean relative survival over all six incubation times of in vitro-digestion.

Six incubation times from 6h rumen liquid + 8h HCl-pepsin to 120h+8h; bars: ± SE; significant differences among species by H-Test; different letters indicate significant differences between species at $p<0.05$ by U-Test; abbreviations for plant species see Table 4.1.

Even after a total incubation time of 128h (120h rumen liquid + 8h HCl-pepsin) viable diaspores of five of the twelve exposed species could be found (Fig. 4.10). After 20h (12h+8h), more than 90% of the incubated viable *Achillea millefolium* and *Poa angustifolia*-diaspores were still unharmed (Fig 4.10). Whereas viability of *Poa angustifolia*-diaspores decreased only slowly with increasing incubation time, those of *Achillea millefolium* declined rapidly after a simulated digestion time of 30h. Most other species showed a more or less continuous decline of viability. Remarkably, survival of both legumes (*Anthyllis vulneraria, Lotus corniculatus*) remained more or less constant during all incubation times, yet on quite different levels. A comparison of the proportion of remaining hard diaspores with those of the control shows that the proportion of hard diaspores after each in vitro-digestion time differs not from the one of untreated diaspores ($p<0.01$, U-Test).

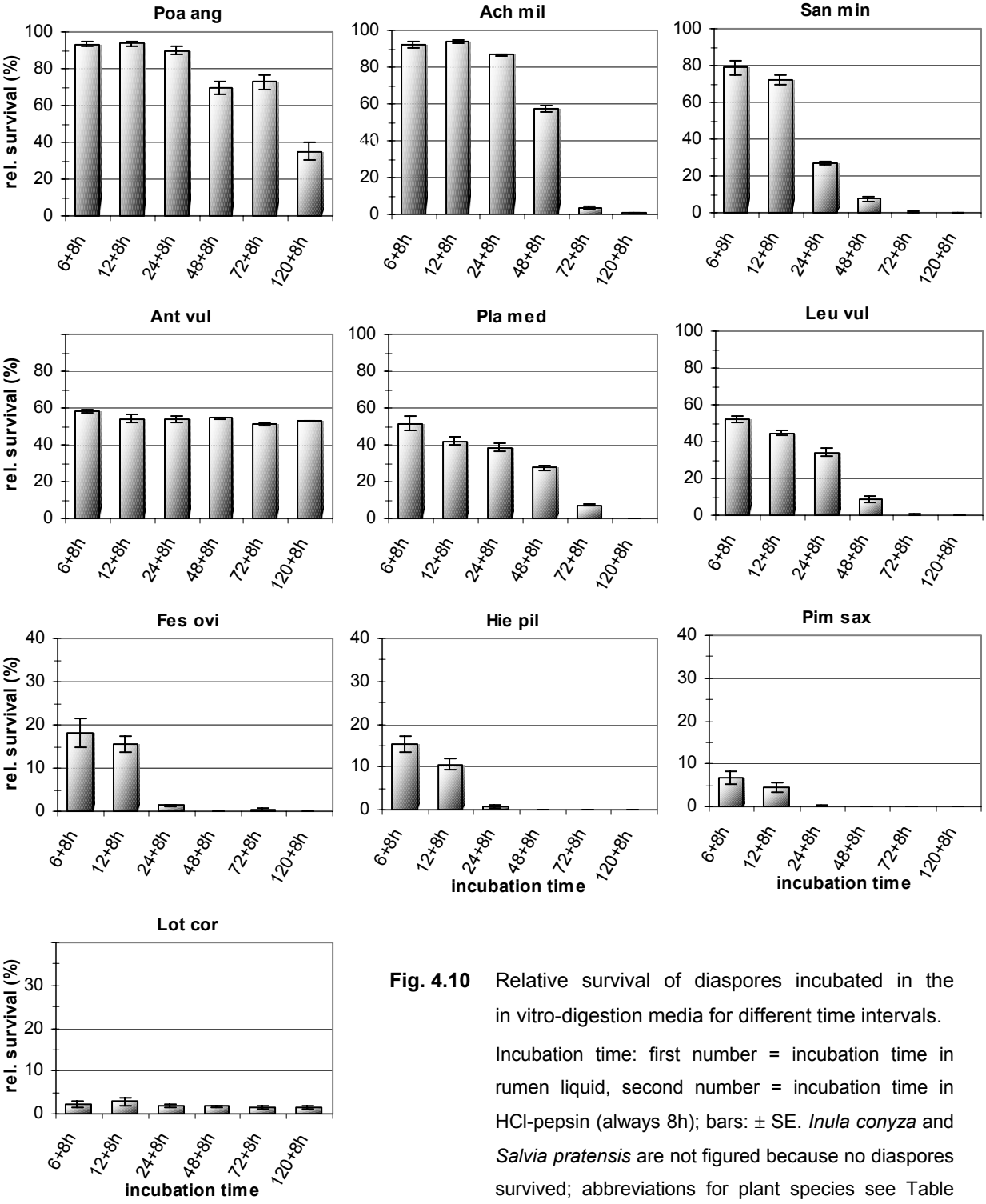


Fig. 4.10 Relative survival of diaspores incubated in the in vitro-digestion media for different time intervals. Incubation time: first number = incubation time in rumen liquid, second number = incubation time in HCl-pepsin (always 8h); bars: ± SE. *Inula conyza* and *Salvia pratensis* are not figured because no diaspores survived; abbreviations for plant species see Table 4.1.

Pearson's correlation coefficient gave no significant correlations between in vitro-survival rates and any morphological diaspore attribute (length, width, height, shape, characteristics of the seed coat, s. chap. 5).

Effect of diaspore incubation in anoxic water and subsequent immersion in HCl

Averaging mean survival rates of all six incubation times in anoxic water followed by an incubation in 0.1 M HCl (Fig. 4.11), reduction of viability varied greatly among the 12 species. Except for *Anthyllis vulneraria* and *Festuca ovina* as well as *Inula conyza* and *Salvia pratensis,* mean survival of all species differed (U-Test, $p<0.05$). *Achillea millefolium* survived the treatment best with a mean viability of 93.5%, followed by *Plantago media* and *Poa angustifolia* with a mean viability >80% in relation to untreated diaspores. Again, *Inula conyza* and *Salvia pratensis* survived worst, but in contrast to most other treatments, few diaspores of both species were viable after single incubation times.

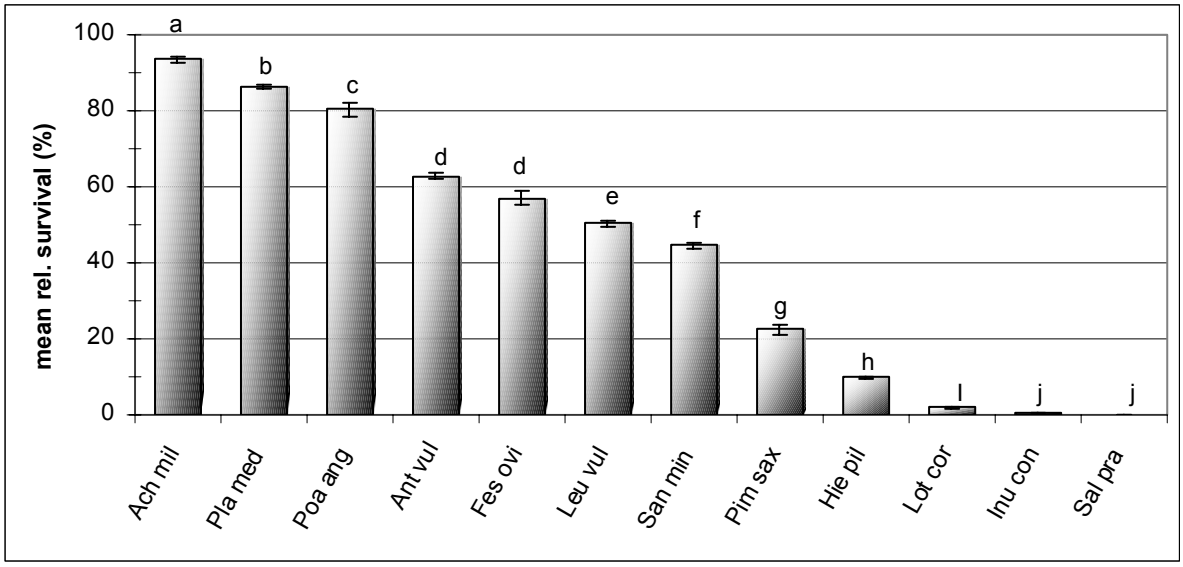


Fig. 4.11 Mean relative survival over all six incubation times in anoxic water (6h to 120h) followed by 8h in 0.1 M HCl.

Bars: ± SE; significant differences among species by H-Test; different letters indicate significant differences between species at $p<0.05$ by U-Test; abbreviations for plant species see Table 4.1.

Illustrating diaspore survival as a function of incubation time in anoxic water, it becomes obvious that viability remained more or less constant over all incubation times for most species (Fig. 4.12). Except for *Poa angustifolia*, viability of all species was reduced in relation to the control by an incubation in anoxic water for 6h followed by an immersion in HCl for 8h (U-Test, $p<0.05$). For *Poa angustifolia*, a reduction of viability in relation to untreated diaspores was reached after 12h+8h. After the initial reduction of viability further losses of viable diaspores could only be observed for *Anthyllis vulneraria* (after 48h+8h) and *Leucanthemum vulgare* (after 12h+8h). After a further reduction of relative diaspore viability after 24h, viability of *Leucanthemum vulgare* remained constant according to the other species. The same is true for *Anthyllis vulneraria* after 72h+8h. For

Anthyllis, it was again the number of soft diaspores which declined with increasing incubation time, whereas the number of hard diaspores remained constant (U-Test, $p<0.01$).

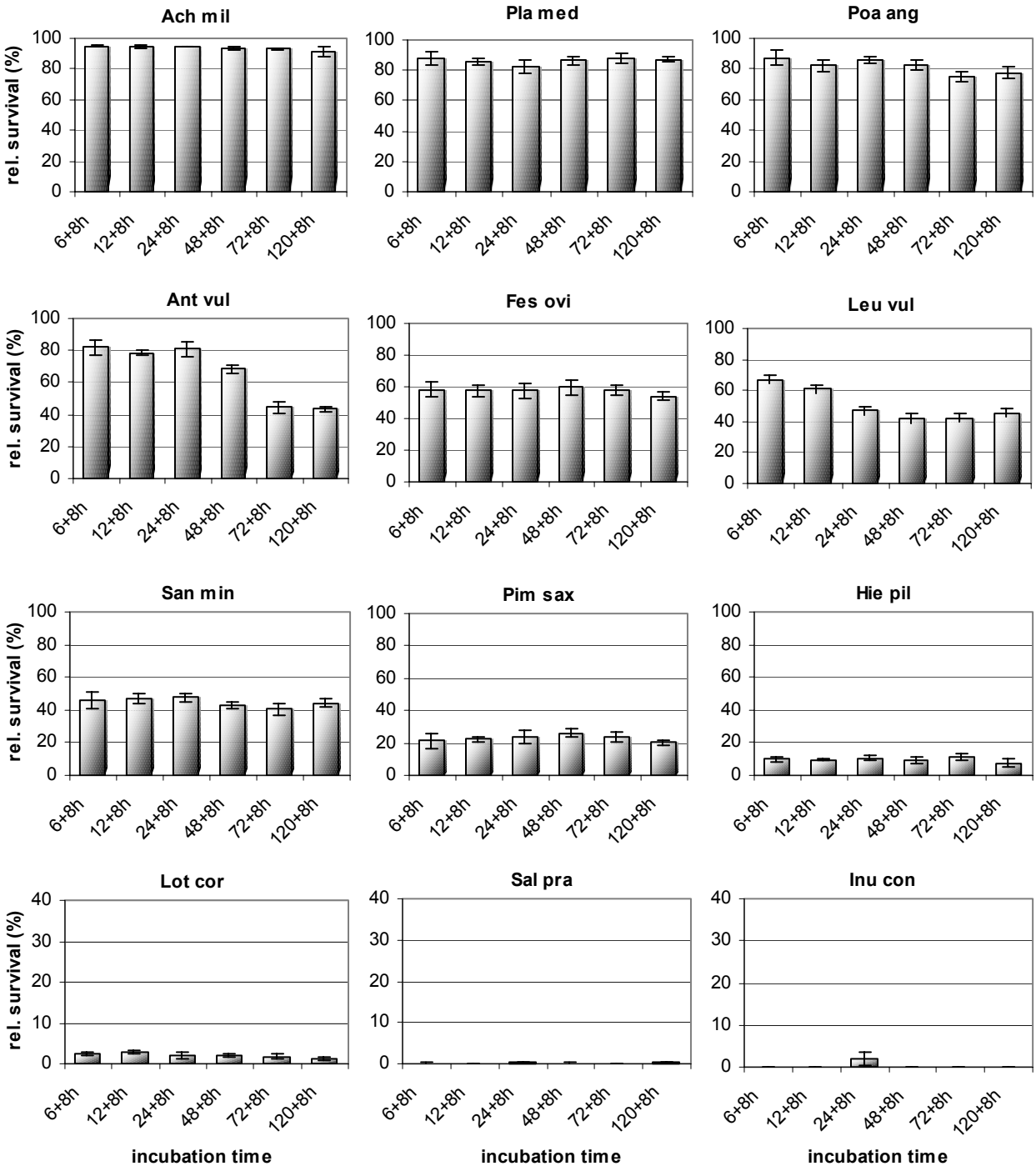


Fig. 4.12 Relative survival of diaspores after an incubation in anoxic water for different time intervals (6h to 120h) followed by an incubation in 0.1 M HCl for 8h.

Bars: ± SE; abbreviations for plant species see Table 4.1.

A comparison of survival rates after a mere immersion in HCl for 8h and an immersion in HCl with a preceding incubation in anoxic water gave for most species no difference. However, after 48h in anoxic water followed by 8h acid immersion, viability of *Leucanthemum vulgare* was lower than after a mere acid treatment for 8h, viability of *Anthyllis vulneraria* after 72h+8h (U-Test, $p<0.05$). *Festuca ovina* and *Plantago media* had even **higher** survival rates after an incubation in anoxic water followed by an immersion in acid (U-Test, $p<0.05$). The higher survival of both species after a combination of an immersion in anoxic water and HCl accounted for all six incubation times (6h to 120h, each followed by an incubation in HCl for 8h; U-Test, $p<0.05$).

Methods to simulate diaspore passage through the digestive tract of ruminants – synopsis

All approaches to simulate various parts of the digestive system of ruminants led to different reactions of the exposed species with respect to diaspore viability. Qualification of the used methods for simulating the effect of ruminant digestion were tested by Pearson's correlation between the relative germination of the diaspores after passing the digestive tract of cattle and sheep and the relative survival of diaspores after the different treatments (Table 4.8, 4.9).

Table 4.8 Correlations between relative germination of diaspores after passing the digestive tract of sheep (in vivo-digestion) and relative diaspore survival after different approaches simulating the passage through the digestive tract (simulated chewing followed by an immersion in HCl; in vitro-digestion; incubation in anoxic water followed by an immersion in HCl; mere immersion in HCl).

Pearson's correlation coefficient; all data were arcsine-square-root transformed; ** = $p<0.01$; * = $p<0.05$; n.s. = not significant. For in vitro-digestion and incubation in anoxic water mean relative survival over all six incubation times was used for calculation.

treatments & intensities		correlation coefficients with relative germination
step 1	**step 2**	**of diaspores fed to sheep**
rumen fluid	**acidified pepsin – 8h**	0.80**
2x 90°	**0.1 M HCl – 8h**	0.79**
0.1 M HCl – 8h	**–**	0.75**
anoxic water	**0.1 M HCl – 8h**	0.75**
2x 90°	**0.1 M HCl – 2h**	0.70*
4x 90°	**0.1 M HCl – 8h**	0.69*
4x 90°	**0.1 M HCl – 2h**	0.64*
4x 90°	**–**	0.63*
2x 90°	**–**	0.57 n.s.
0.1 M HCl – 2h	**–**	0.56 n.s.

Table 4.9 Correlations between relative germination of diaspores after passing the digestive tract of cattle (in vivo-digestion) and relative diaspore survival after different approaches simulating the passage through the digestive tract (simulated chewing followed by an immersion in HCl; in vitro-digestion; incubation in anoxic water followed by an immersion in HCl; mere immersion in HCl).

Pearson's correlation coefficient; all data were arcsine-square-root transformed; ** = p<0.01; * = p<0.05; n.s. = not significant. For in vitro-digestion and incubation in anoxic water mean relative survival over all six incubation times was used for calculation.

treatments & intensities		correlation coefficients with relative germination
step 1	**step 2**	**of diaspores fed to cattle**
rumen fluid	**acidified pepsin – 8h**	0.77**
0.1 M HCl – 8h	**–**	0.75**
2x 90°	**0.1 M HCl – 8h**	0.72**
2x 90°	**0.1 M HCl – 2h**	0.65*
anoxic water	**0.1 M HCl – 8h**	0.64*
4x 90°	**0.1 M HCl – 8h**	0.63*
0.1 M HCl – 2h	**–**	0.58*
4x 90°	**0.1 M HCl – 2h**	0.57 n.s.
4x 90°	**–**	0.55 n.s.
2x 90°	**–**	0.53 n.s.

Relative survival after in vitro-digestion with rumen fluid followed by an immersion in acidified pepsin correlated best with relative survival after in vivo-digestion by sheep as well as by cattle. However, compared to diaspores germinating in the dung of sheep, simulated chewing (2x 90°) followed by an immersion in hydrochloric acid for 8h gave only a marginally poorer correlation (Table 4.8). For cattle, an immersion in 0.1M HCl for 8h gave the second best result. An additional chewing (2x 90°) led to the third best, still high significant (p<0.01) correlation.

Discussion

Survival of diaspores from different plant species fed to cattle and sheep

As shown in previous feeding experiments (e.g. ATKESON et al. 1934; LENNARTZ 1955, 1957; GARDENER et al. 1993a; IDE et al. 1999), there are big differences in survival capacity among plant species, even between those of the same plant family, when passing the ruminants digestive system. In the present study, diaspore feeding to cattle and sheep gave similar results with respect to total diaspore germination in the exposed dung. Other studies showed that survival rates also differ among ruminants: passing the digestive system of sheep often proved to be more destructive for ingested diaspores than the one of cattle (e.g. HARMON & KEIM 1934; THILL et al. 1986; SIMAO

NETO et al. 1987; KNEUPER et al. 2003). These differences seem not to be caused by different effects of the digestive fluids, since SIMAO NETO et al. (1987) showed that the digestion of a milled basal diet was identical for both animal species. They therefore interpreted the lower survival rates after passing the gut of sheep as a result of mastication and rumination. Sheep have a smaller orifice (POPPI et al. 1985), which requires a more intense comminution of feed (ULYATT et al. 1986). In the present study, at least the lower survival rates of *Sanguisorba minor*, the species with the largest diaspores, after passing the digestive tract of sheep confirms this hypothesis. Additionally, diaspore survival after digestion by cattle might be slightly underestimated in the present study, because cattle dung was excreted on straw, which made an entire dung collection more difficult. Nevertheless, comparisons among plant species regarding their survival capacity when passing the digestive tract of cattle were correctly estimated.

An underestimation of viable diaspores excreted might also be caused by the time span of dung collection, which, however, concerns both animal species similarly. Several plant species were represented in the dung samples already at the beginning of dung collection 12h (sheep) and 17h (cattle) after diaspore feeding, respectively (Fig. 4.4, 4.5). BONN & POSCHLOD (1998a) gave minimum retention times of 6h for sheep and 8h to 10h for cattle. Although most species were excreted only by individual animals, an orientation on these minimum retention times would have led to a more accurate estimation of total survival rate and is therefore recommended for future experiments. For cattle, the finishing of dung collection after 8 days also proved as slightly untimely, because faeces contained still few viable diaspores. Excretion of viable diaspores by sheep was finished six days post feeding.

As in previous studies (NORTON et al 1989; GARDENER et al. 1993a; DOUCETTE et al. 2001) survival of legumes was related to the proportion of hard seeds. However, the proportion of hard and soft diaspores germinating in the excreted dung was quite different for both legume species. Of 2268 hard *Anthyllis vulneraria*-seeds fed to each ruminant, not even half germinated (1018 seedlings in cattle dung, 776 in sheep dung). In contrast, germination of *Lotus corniculatus* (0.6% hard seeds) in dung was three (cattle) to four (sheep) times higher than the number of hard seeds fed. Therefore, soft seeds of *Lotus* must also have survived the passage of the digestive tract. Similarly, GARDENER et al. (1993a,b) showed that there may be a high interspecific variability in the proportion of hard diaspores disintegrating during the passage of the digestive tract. NORTON et al. (1989) even documented big differences between different lines of the same plant species. Furthermore, previous studies confirm that soft seeds of some species may also survive the passage of the digestive tract (GARDENER et al 1993a). The survival capacity of soft legume diaspores with a highly permeable seed coat illustrates, that species surviving the passage through the digestion tract must not necessarily be equipped with an impermeable seed or fruit coat (see also KIRK & COURTNEY 1972). However, survival rates of such species will always be low. In the present study, may be true e.g. for *Hieracium pilosella* or *Pimpinella saxifraga*.

Obviously, documented survival was only partly caused by the damage of diaspores during the passage of the digestive tract, because germination of undigested diaspores exposed on dung was much lower than viability under controlled conditions in the climate chamber (Fig. 4.3).

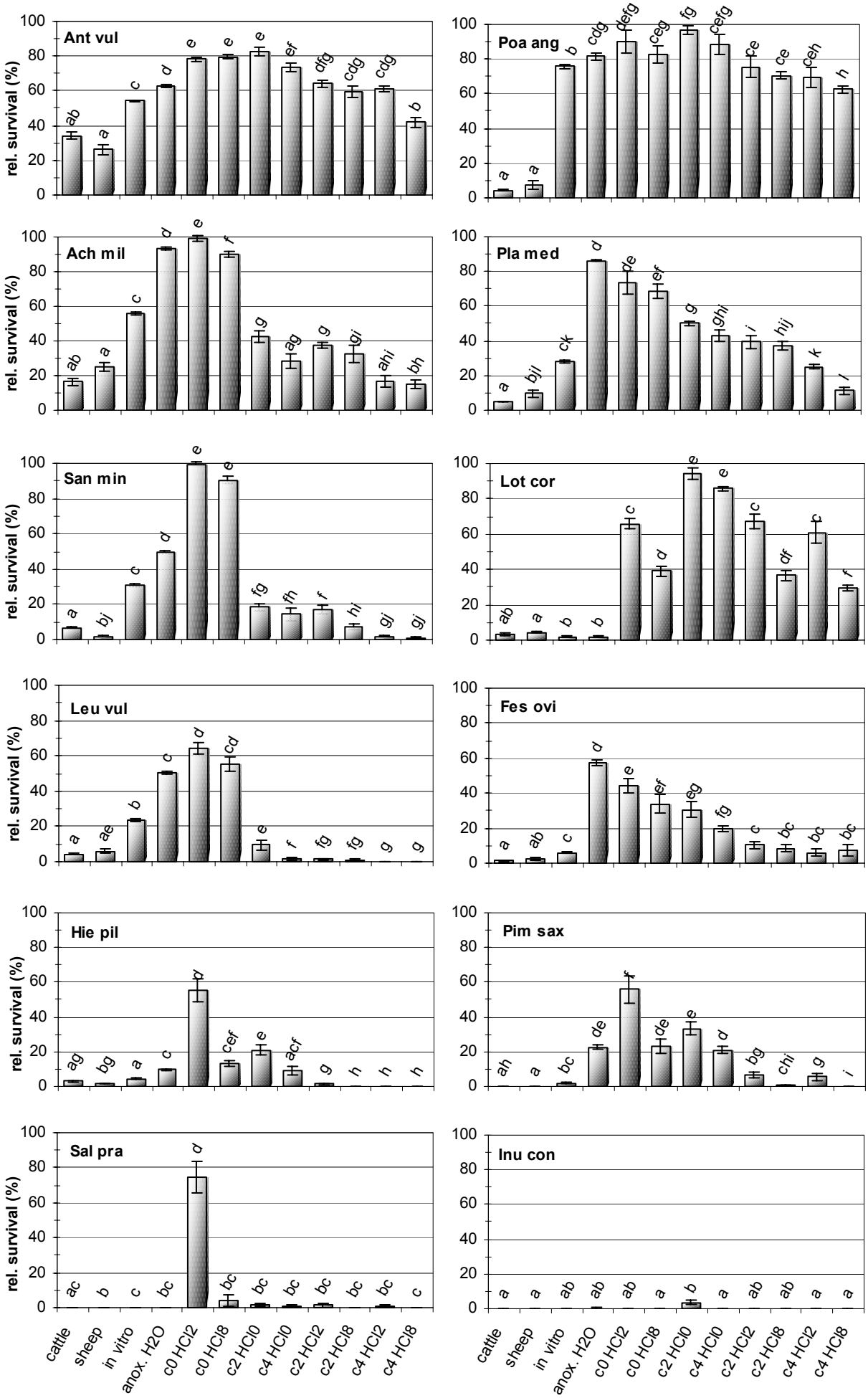
Diaspore exposition in the botanical garden was doubtlessly not as controlled and optimal as in the climate chamber. However, as dung samples in the botanical garden were regularly watered, at least part of the reduced germination on the dung samples must be attributed to the biotic and abiotic conditions in the dung. Residues of bacteria from the ruminant gut are present in the faeces (VAN SOEST 1994). Consequently, SIMAO NETO & JONES (1986) documented a loss of viability when diaspores were stored in cattle dung, whereas GHASSALI et al. (1998) could find no differences between germination rates of naked diaspores and diaspores exposed in faecal pellets of sheep. Due to the bacteria still present in faeces, EL SHAER et al. (1987) even showed that digestibility of feed determined in vitro with inoculated sheep faeces correlates closely with in vivo-digestibility (see also AIPLE 1993; JONES & BARNES 1996). Although the used dung was not freshly voided, remnants of the typical faecal organisms may have been still present. Germination on dung was correlated with diaspore viability determined in the climate chamber ($r=0.71$, $p<0.01$, Pearson's correlation coefficient), but not with the germination of diaspores after passing the ruminant gut. In contrast to in vivo-digestion, which is selective with respect to mastication and particle retention (ULYATT et al. 1986; KASKE & ENGELHARDT 1990), effects of the faecal environment on diaspore germination are unselective and therefore affect all species similarly and in general not as much as in vivo-digestion.

Approaches to simulate the ruminant digestive system

The different approaches to simulate the ruminant digestive system were based on chemical, microbial and/or mechanical treatments of the diaspores. All these approaches affected diaspore viability of most of the tested plant species (Fig. 4.13), although viability of only few species was reduced as much as by the in vivo-digestion process. Especially those species with very low or no survival after the passage of the digestive tract, *Hieracium pilosella*, *Inula conyza, Pimpinella saxifraga* and *Salvia pratensis*, reacted similar sensitive to nearly all laboratory treatments, indicating a highly permeable and/or fragile seed coat*. For these species, a relative survival of 50% and more could be only observed after the lower intensities of either acid or mechanical treatment (Fig. 4.13).

Fig. 4.13 **(next page)** Relative survival of plant species after in vivo-digestion by cattle and sheep and after different approaches to simulate the passage through the digestive tract (in vitro-digestion (in vitro), bath in anoxic water followed by 8h HCl treatment (anox. H2O), simulated chewing (c0=without simulated chewing, c2=2x simulated chewing, c4=4x simulated chewing) and/or acid treatment (HCl0=without HCl, HCl2=2h in HCl, HCl8=8h in HCl)).
Bars: ± SE; different letters indicate significant differences in survival at $p<0.05$ by U-Test; diaspores of *Sanguisorba minor* and *Lotus corniculatus* exposed in anoxic water and subsequently in HCl were not of the same origin than the diaspores used for all other experiments.

* The term seed coat is used for the embryo surrounding structures, although several plant families, like Poaceae and Asteraceae, have a pericarp instead of a seed coat.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JANZEN et al. (1985) already tested the influence of **anoxic water** in relation to other in vitro-digestion techniques on the germination of hard and scarified seeds of *Enterolobium cyclocarpum* (Fabaceae) and concluded that "an anaerobic environment [water] alone is only marginally safer than the inoculated solution". In the present study, however, most species showed similar viability after an incubation in anaerobic water followed by another incubation in HCl for 8h and after a mere immersion in (aerobic) HCl for 8h (Fig. 4.13). *Plantago media* and *Festuca ovina* even survived the combination of the anoxic fluid with subsequent HCl-immersion better than a mere immersion in acid. Only two species, *Anthyllis vulneraria* and *Leucanthemum vulgare,* showed after 48h and 72h respectively, each followed by 8h acid immersion, a lower viability. Despite the mostly similar survival after an incubation in anaerobic water followed by an immersion in HCl and after a mere immersion in HCl, seed coats of several species, as those of *Anthyllis*, *Hieracium, Inula, Leucanthemum*, *Lotus* and *Pimpinella,* were ruptured already after the incubation in the anoxic solution. Although the seed coats of the other species remained intact, they imbibed water, which was documented by weighing them before and after the incubation (unpublished data). Similarly, GARDENER et al. (1993b) described that the seed coats of various legumes ruptured in anoxic water, whereas the caryopses of various grass species imbibed less and were not visibly swollen. For the latter, GARDENER et al. (1993a,b) postulated that they become functionally dormant in the digestive tract (see also FREDRICKSON et al. 1997). Although they imbibe, germination is arrested under anoxic conditions (CORBINEAU & CÔME 1995) and the pericarp may act as a barrier against enzymes, acids and microbes (GARDENER et al. 1993a). In the present study this might explain the higher viability of *Festuca* and *Plantago* after combining imbibition in anaerobic water with an acid bath in relation to a mere immersion in HCl. Contrary to aerobic conditions, as in the acid bath, where species imbibe continuously and may even germinate (GARDENER et al. 1993b), they imbibe under anoxic conditions less and built up a barrier, which still prevents an entering of the acid during the subsequent aerobic immersion in HCl. Similar survival rates of a species after anoxic imbibition followed by an immersion in HCl and a mere acid bath may be caused by a species specific amount of diaspores imbibing quickly, regardless of the presence of oxygen. For the remaining diaspores, the lack of oxygen may prevent imbibition and even a negative effect of the subsequent aerobic acid treatment.

Corresponding to studies of OCUMPAUGH & SWAKON (1993), who proposed in vitro-digestion as a standard method to assess endozoochorous dispersal potential, relative **diaspore survival after in vitro-digestion** resulted in the present study in the best correlations with in vivo-digestion (Fig. 4.14, Table 4.8, 4.9). However, survival rates after in vitro-digestion were mostly higher than survival after passing the alimentary tract of ruminants. Survival rates of *Poa angustifolia* deviated most from survival after passing the alimentary tract, because of its low survival after in vivo- and high survival after in vitro-digestion (Fig. 4.13).

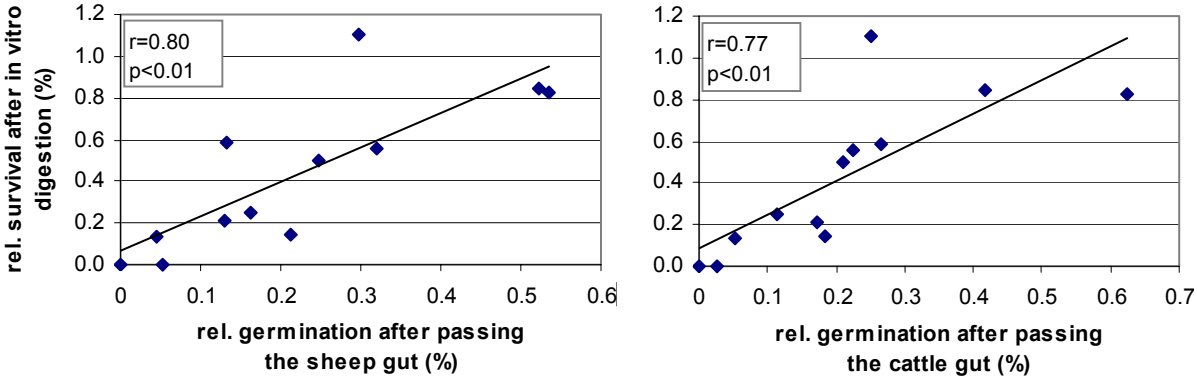


Fig. 4.14 Correlations between relative germination of diaspores after passing the alimentary tract of sheep or cattle and relative survival after in vitro-digestion.

Pearson's correlation coefficient; all data were arcsine-square-root transformed.

In relation to imbibition in anoxic water all species except of *Lotus corniculatus* as well as *Inula conyza* and *Salvia pratensis* (both in general with only few surviving diaspores) showed lower survival rates after anoxic in vitro-digestion with ruminal fluid followed by acidified pepsin (Fig. 4.13). Except for *Achillea millefolium, Hieracium pilosella, Poa angustifolia* and *Sanguisorba minor* lower survival rates were already observed after the shortest incubation time (6h water/rumen fluid followed by 8h HCl/HCl-pepsin) (Fig. 4.8, 4.10). The additive presence of ruminal bacteria and enzymes in the anoxic fluid obviously affects diaspore viability of most species. Ruminal bacteria are very effective cellulose and fibre-digesting organisms (WINDHAM & AKIN 1984) and may degrade the testa of a diaspore, allowing imbibition (HOWARD & ELLIOTT 1988). However, the relevance of the different digestive fluids and the influence of previous mastication for diaspore survival seems to be strongly diaspore specific: According to SIMAO NETO & JONES (1987) most of the damage of ingested diaspores is caused by the microbial activity in the rumen and some by enzymes in the abomasum. In contrast, OCUMPAUGH & SWAKON (1993) showed for various grass species that ruminal digestion alone reduces viability only slightly, if it is not followed by an acid-pepsin treatment. BEAUCHEMIN et al. (1994) documented a species-specific dry-matter disappearance of various intact cereal grain when placed in the rumen (see also FREDRICKSON et al. 1997). A dry matter disappearance could be measured for wheat after an incubation time of 20h, but for corn and barley immediately after incubation. IBANEZ & PASSERA (1997) compared the effect of rumen fluid on *Anthyllis cytisoides*-diaspores by incubating diaspores into the rumen of sheep via ruminal fistulae with a chemical scarification by different acids and a mechanical scarification with sandpaper. In contrast to the acid and mechanical treatments, germination success after incubation in ruminal fluid differed not from the control, although germination was fastened. NORTON et al. (1989) hypothesize that mastication and not digestion itself is the main reason for the loss of the hard seeds of legumes. Correspondingly, MCALLISTER & CHENG (1996) postulate for cereal grain, that the pericarp is resistant against microbial attachment and penetration if it is not previously fractured by mastication (see also BEAUCHEMIN et al. 1994; QUINN et al. 1994). Following

PALMQUIST (1995), the role of a previous mastication depends on the degree of lignification of the seed coat, because ruminal bacteria are extensively unable to degrade lignins.

Whereas viability of both grass species was reduced in the present study during in vitro-digestion, the constant number of hard diaspores similar to the one of the control (see above) confirms the results from previous studies for legumes (NORTON et al. 1989). A comparison with in vivo-survival rates shows that on the one hand, hard diaspores obviously have been digested due to previous scarification by mastication (*Anthyllis vulneraria*). The lack of mastication may also explain the higher survival of most other species during in vitro-digestion. On the other hand, also soft legume (*Lotus corniculatus*) diaspores were able to survive in vitro-digestion, as it was already observed for in vivo-digestion. Correspondingly, JANZEN et al. (1985) showed for scarified legume diaspores that after an incubation time of one day in the rumen 11% survived, whereas after two days, all diaspores were killed. In the present study, the number of soft *Lotus*-diaspores was drastically reduced even after 6h in rumen fluid followed by 8h acid pepsin. Although the number of germinating diaspores declined continuously, a further significant reduction of the number of soft germinating diaspores was observed not before 72h in rumen fluid plus 8h acidified pepsin. Obviously, even diaspores with a permeable seed coat are able to survive hours to days in the digestive juices. Even grass caryopsis where the distal end was clipped off survived an incubation of 48h in the rumen (GARDENER et al. 1993b). Although this study only included ruminal fluid without considering the following acid-pepsin digestion, it illustrates that even species with a permeable seed coat, a permeable mikropyle or those that are scarified by chewing may survive the gut passage, if they pass quick enough. This was also shown by scarified as well as dehusked diaspores of *Avena fatua* and *A. sativa,* which were able to survive the passage through the digestion tract of cattle (KIRK & COURTNEY 1972).

For several legume and grass species it was shown that mechanical, chemical and microbial stress may substitute each other. E.g. GARDENER et al. (1993b) suppose that a missing mechanical damage by chewing can be balanced by a longer period in an acid medium as acidified pepsin. In the present study the **simulation of mastication and the chemical attacks in the abomasum** showed that, except for *Poa angustifolia*, a chemical as well as mechanical treatment alone already reduced viability of all species. Especially an acid treatment for 8 hours correlated highly significant with survival rates after in vivo-digestion (Table 4.8, 4.9). Correlations of total survival rates after in vitro-digestion (rumen fluid followed by HCl-pepsin) with survival after mechanical stress ($r=0.78$, $p<0.01$ for 2x chewing + 8h HCl; $r=0.72$, $p<0.01$ for 4x chewing + 8h HCl, Pearson's correlation coefficient) confirm that the different treatments including either microbial and chemical or mechanical and chemical stress have similar effects on species survival, although on different levels (Fig. 4.13).

Again, earlier studies concerning the effect of a chemical or mechanical treatment on diaspore germination and viability concentrated predominantly on legumes and grasses. Results of these studies were quite variable and confirm the results of the present study showing interspecific differences in the tolerance of chemical and mechanical strain. SIMAO NETO & JONES (1987) found

little effect of an acid (2.42 M HCl)-pepsin treatment of different Poaceae without a preceding exposure of the diaspores in rumen fluid (see also OCUMPAUGH & SWAKON 1993). Similarly, a treatment of cacti-diaspores with HCl (pH 1) increased germination only in one of eight exposed species (GORDINEZ-ALVAREZ & VALIENTE-BANUET 1998). However, TISCHLER & YOUNG (1983) indicated that a treatment of *Panicum coloratum*-diaspores with sulphuric acid increased germination by breaking post-harvest dormancy (see also WEST & MAROUSKY 1989) and diaspores were even injured, if exposed too long. BAES et al. (2002) showed for *Prosopis ferox*, a hard seeded legume, that a chemical scarification with sulphuric acid (98%, 3 min exposure) and a mechanical scarification by an abrasion with sand paper led to high germination rates (see also FULBRIGHT & FLENNIKEN (1987) and IBANEZ & PASSERA (1997) for other legumes). A chemical scarification with HCl (37%, 3 min exposure) resulted in lower germination rates, which were, however, still higher than after the biological scarification via the passage of the digestive tract of goats and donkeys. An additional mechanical scarification of ungerminated seeds which had passed the ungulates gut led to germination rates similar to those after chemical or mechanical scarification. ROTH et al. (1987) found also an increase in germination of three legume species after mechanical scarification. However, all seedlings died soon after germination because the embryo was damaged. Germination of *Robina pseudacacia* diaspores was even reduced by mechanical scarification (GRUNICKE 1996). All these studies show that both, mechanical and chemical treatment, can scarify the testa, allowing imbibition and germination, but they may also injure or kill the embryo. In the rumen, a seed coat or pericarp damaged by mastication or by microbial attacks allows ruminal bacteria as well as postruminal acids and enzymes access to the embryo.

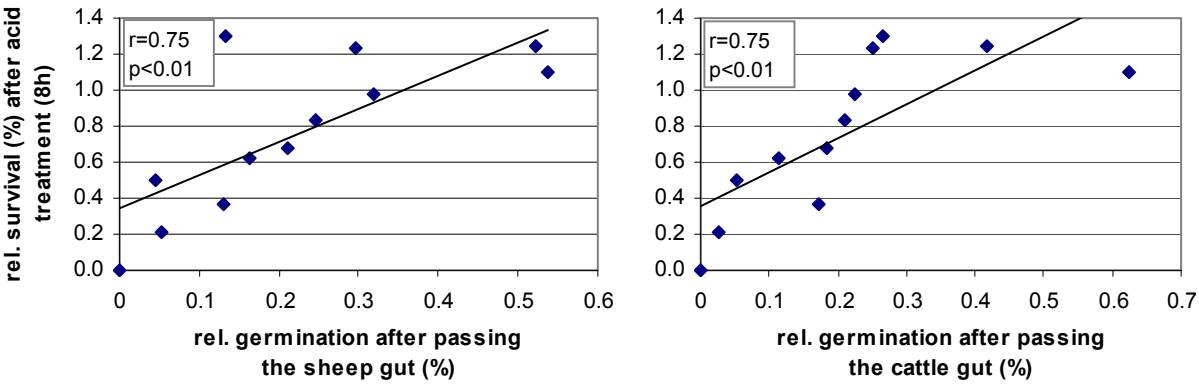


Fig. 4.15 Correlations between relative germination of diaspores after passing the alimentary tract of sheep or cattle and relative survival after an immersion in 0.1 M HCl for 8h.
Pearson's correlation coefficient; all data were arcsine-square-root transformed.

Low correlation coefficients between simulated chewing without a subsequent acid-treatment and diaspore germination after the passage of the digestive tract (Table 4.8, 4.9) indicate that a mere mechanical stress is insufficient to simulate survival capacity. This may be based on the fact

that mastication and rumination are in vivo always followed by ruminal and abomasal digestion. Although a diaspore, especially a small one (DESWYSEN & ELLIS 1990), may escape the mechanical attacks in the mouth, it always has to pass the rumen, abomasum and duodenum of a ruminant. In contrast, a mere treatment with 0.1 M HCl for 8h gave the second best correlations with diaspore survival after passing the alimentary tract of cattle (Table 4.9, Fig. 4.15), although survival rates after the acid treatment were markedly higher than those after gut passage. Survival rates after diaspore digestion by sheep correlated best with a combination of simulated chewing (2x) and an acid bath of 8h (Fig. 4.16, Table 4.8). This might be attributed to the fact that sheep are chewing more intensely because of their smaller orifice (POPPI et al. 1985), requiring a more intense comminution of feed (ULYATT et al. 1986, see also OWENS & GOETSCH 1988). However, if *Poa angustifolia*, in which in vivo-survival rates differed considerably from all experimental approaches, is excluded from the correlation analysis, simulated chewing (2x) and an acid bath for 8h correlates also second best with survival rates after digestion by cattle.

Proposal of a standardized method to simulate the passage of the digestive tract of ruminants

Although in vitro-digestion in ruminal fluid followed by an immersion in acid pepsin gave the highest correlation with in vivo-survival rates (Table 4.8, 4.9), this method is rejected with regard to large-scale standardized measurements. An inherent disadvantage of this technique lies in the dependence on a continuous supply with rumen inoculum, requiring access to animals fitted with rumen canulae. Furthermore, alternative approaches had only slightly lower correlation coefficients (Table 4.8, 4.9). A mere treatment with 0.1 M HCl for 8h, which gave the second best correlation with survival after passing the cattle gut (Table 4.9), is judged as not qualified as a standard method, because survival rates were much higher than after in vivo-digestion (Fig 4.13). **Therefore, simulated chewing (2x) followed by a bath in 0.1 M HCl for 8h is proposed as a standard method for the assessment of endozoochorous dispersal potential of diaspores**. Because sheep usually destroy more diaspores during digestion than cattle do (see above), a method correlating best with survival rates after passing the sheep gut will give a good minimal estimation for survival potential. Similarly, SIMAO NETO et al. (1987) and FREDRICKSON et al. (1997) judged that sheep provide a considerable more conservative estimate of a species survival capacity when passing the ruminants guts. However, since germination rates proved always to be extremely variable, a higher number of replicates (10) than used in the present study (5) is advisable.

For legumes a determination of the proportion of hard diaspores should be used as an alternative method for the assessment of endozoochorous dispersal potential (see also GARDENER et al. 1993b). This method may be also applied for other species with physical dormancy, as it is characteristic for example of the Cistaceae or Convolvulaceae (BASKIN & BASKIN 1998; BASKIN et al. 2000).

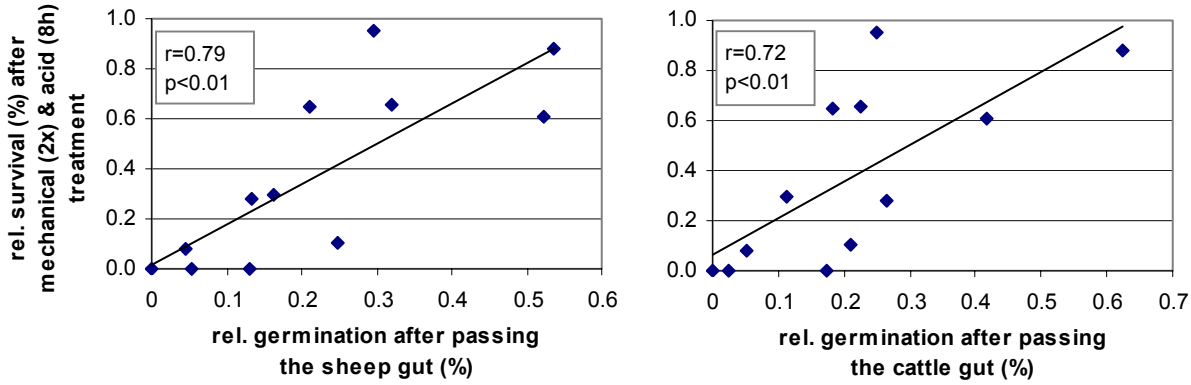


Fig. 4.16 Correlations between relative germination of diaspores after passing the alimentary tract of sheep or cattle and relative survival after a mechanical treatment (2x simulated chewing) followed by an immersion in 0.1 M HCl for 8h.

Pearson's correlation coefficient; all data were arcsine-square-root transformed.

Assessment of endozoochorous dispersal potential

An assessment of gradual differences in the dispersal potential of plant species is a basic tool for the conservation and restoration of species rich communities and the risk assessment of plant species (HUSBAND & BARRETT 1996; EHRLÉN & VAN GROENENDAEL 1998; BONN et al. 2000; POSCHLOD et al. 1999, 2000; TACKENBERG et al. 2003). However, the grade of specification of a species' dispersal potential by a certain vector has been only rarely discussed. For anemochory, TACKENBERG et al. (2003) proposed a nine-graded classification system based on dispersal distances. A corresponding detailed classification system is not suitable for endozoochory. In contrast to wind, dispersal by animals follows no distinct physical rules determining dispersal distance. If an ingested diaspore has the potential to survive the trip through the digestive tract of ruminants, it also has a potential for long distance dispersal. A minimum retention time of several hours (see above, BONN & POSCHLOD 1998a) and a minimum time for 50% diaspore excretion of 14h (Table 4.7) are long enough for a ruminant to bridge several kilometers, if it is allowed to do so.

In contrast to other dispersal modes, as anemo-, hydro- or epizoochory, where diaspores of all herbaceous species have the principle chance of being dispersed (e.g. KELLY & BRUNS 1975; FISCHER et al. 1996; TACKENBERG 2001) diaspore dispersal via passing the digestive tract of mammals is selective. For herbaceous species, grazing mammals represent predators and dispersal vectors at the same time (JANZEN 1984; COLLINS & UNO 1985). Species are completely excluded from this mode of dispersal if they are incapable to survive the trip through the gut. In the present study this could be shown for *Inula conyza* and *Jasione montana*. Additionally, both species have never been found in dung (source: DIASPORUS, BONN et al. 2000, chap. 3), which manifests the results of the present study, indicating they have no or at least a negligible endzoochorous dispersal potential. Therefore, the simple traditional allocation of species as "dispersed" versus "not dispersed" to a certain dispersal mode (e.g. LUFTENSTEINER 1982; GRIME et al. 1988), which has been proved as untenable for the above mentioned dispersal modes anemo-,

hydro- or epizoochory (TACKENBERG et al. 2003), would be a rough but – from the present state of knowledge – already helpful classification of endozoochorous dispersal potential. TACKENBERG (2001) also recommended a two-staged classification of endozoochorous dispersal potential, based on records of a species in the dung of mammals in previous studies (source: DIASPORUS, BONN et al. 2000, chap. 3) and the existence of hard diaspores as a morphological criterion. A two-graded scale (dispersed versus not dispersed) was also used by POSCHLOD et al. (1998).

Although a simple binary system, classifying species as those with or without an endozoochorous dispersal potential is even more than we can perform today, a more detailed classification should be aimed at because interspecific differences in survival potential are remarkable. A four graded classification, for instance, was proposed by POSCHLOD et al. (1997), based on the frequency of germinating diaspores in dung samples collected from grazing sheep, whereas BONN et al. (2000) recommended the use of survival rates (%) after a standardized treatment (see chap. 3).

A too detailed classification of endozoochorous dispersal potential, however, will fail the actual variability of ruminant digestion as well as the intraspecific variations in the stress-tolerance of diaspores. Individual animals may differ greatly with regard to the number of diaspores surviving the gut passage (e.g. BURTON & ANDREWS 1948; RUSSI et al. 1992). This is not only a function of diet quality or level of feed intake, both influencing the retention time (ALWASH & THOMAS 1974; DHIMAN et al. 1995; JIANG & HUDSON 1996) and therefore the intensity of digestion (ALWASH & THOMAS 1974; JONES & SIMAO NETO 1987). Individual animals of the same species also differ in their efficiency to digest feed (DESWYSEN & ELLIS 1990). According to BEAUCHEMIN et al. (1994), a large proportion of the differences between individual cattle to digest whole grains results from differences in chewing during eating, which is erratic and varies greatly between the individuals (see also LEE & PEARCE 1984; LUGINBUHL et al. 1989; BEAUCHEMIN 1991). That differences in chewing may be one factor influencing survival capacity of diaspores was demonstrated in the present study (see also NORTON et al. 1989; FREDRICKSON et al. 1997). Because of this variability JONES & SIMAO NETO (1987) concluded that data from single studies are of limited value to derive general conclusions concerning the endozoochorous dispersal potential of single species.

Furthermore, the reduction of diaspore viability is no constant for individual plant species eaten by a certain ruminant. As illustrated for many legumes, individual populations of a species differ in their survival capacity because of a varying number of hard seeds (e.g. JONES & BUNCH 1988; NORTON et al. 1989). Due to these intraspecific variations, SIMAO NETO et al. (1987) postulated that an extrapolation of results from one seed lot of a species to the species in general must be exercised with caution. For other plant families, a variable reaction of diaspores from different populations or even different harvest years is also hypothesized (e.g. ANDERSSON & MILBERG 1998). In the present study we tested, for example, the sensitivity of *Poa angustifolia* from two different provenances to a HCl-treatment of 2h and 8h as well as well as on 2 and 4 simulated chews (see above). Each of the chemical and mechanical treatments led to different survival rates of the diaspores of the two provenances (Fig. 4.17). Similar observations on intraspecific differences were made in studies on diaspore banks (THOMPSON et al. 1997; BAKKER et al. 2000).

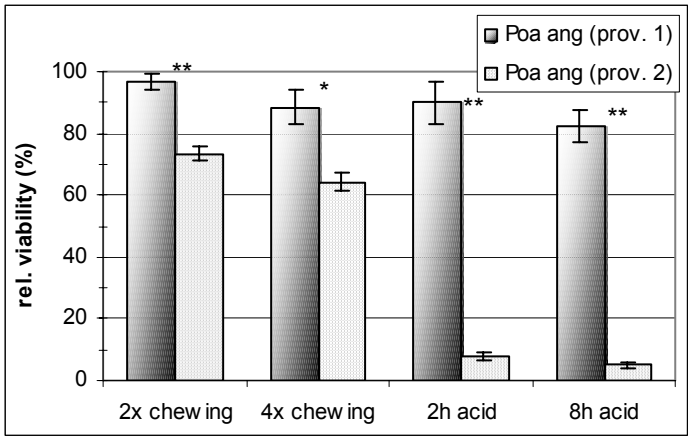


Fig. 4.17 Relative viability (%) of two different provenances (prov. 1/prov. 2) of *Poa angustifolia* after two chemical (2h and 8h in 0.1 M HCl) and two mechanical (2x and 4x chewing) treatments.

Bars: ±SE; **=significant difference at p<0.01, * = significant difference at p<0.05 by U-Test.

Additionally, intraspecific differences in dispersal potential were also observed for anemochory, were different terminal velocities were documented for the same plant species (TACKENBERG et al. 2003). It therefore seems advisable to study different populations of a plant species in order to estimate the intraspecific variability of the endozoochorous dispersal potential of a species.

As a result of the interaction of two living organisms – the animal and the diaspore – endozoochorous dispersal always includes a lot of variability, limiting accuracy of an assessment of endozoochorous dispersal potential. Facing this high variability on the one hand and the helpfulness of a detailed assessment of endozoochorous dispersal potential on the other hand, we recommend a four graded classification with the lowest grade for those species incapable to survive the passage of the digestion tract. Species able to pass the ruminant digestive system unharmed can be classified as those with a low, intermediate and high dispersal potential. The exact limits of these three classes should be set after applying the proposed standardized method to an extensive number of species.

With regard to the application of experimentally assessed data concerning endozoochorous dispersal potential at the landscape level, it has to be considered that the dispersal capacity of a diaspore itself is not the only parameter influencing endozoochorous dispersal potential. BONN et al. (2000) gave a list of plant traits relevant for all dispersal modes, such as diaspore production (see chap. 3). The relevance of this and further plant traits (e.g. palatability) for the assessment of a plant species' endozoochorous dispersal potential will be treated in chapter 5.

Chapter 5 Assessment of endozoochorous dispersal potential of plant species by ruminants – suitability of different plant and diaspore traits

Abstract

The aim of the present study was to identify diaspore or plant traits qualified to predict endozoochorous dispersal potential. Three approaches were used to assess endozoochorous dispersal potential via plant and diaspore traits: 1. two different feeding experiments, 2. data from literature combined with the data of the two feeding experiments in order to obtain a greater number of species and 3. dung samples from grazing herbivores in relation to diaspore supply on the pasture. Diaspore and plant traits tested for their suitability to predict endozoochorous dispersal potential were diaspore morphology (length, width, height, shape), diaspore mass, specific gravity, seed bank longevity index, plant species' palatability and attributes of the seed or fruit coat (thickness, phenol content). Release height and diaspore supply were included as further variables for species germinating in the dung of grazing herbivores or fructifying on the pasture.

Correlations between endozoochorous dispersal potential and the different traits evaluated were quite variable concerning the different approaches. In general, negative correlations were obtained between one of the morphological diaspore traits and relative frequency in herbivore dung. Nearly all data sets of germinated diaspores in the dung of grazing animals showed highest significant correlations with traits relating not to the dispersal unit: release height (negative correlation) and diaspore supply on the pasture (positive correlation). The relevance of diaspore supply was also reflected by discriminant analysis, where this trait proved to be for nearly all data sets the most important discriminating variable. Although various other variables affect endozoochorous dispersal potential, this indicates that the most important variable influencing endozoochory is diaspore supply, which is not only species specific but also influenced by site conditions and, therefore, extremely variable. Using discriminant analysis, however, no transferable statistic models to predict endozoochorous dispersal potential by diaspore or plant traits could be identified.

Introduction

Various morphological characteristics a diaspore are interpreted as evolutionary adaptations to improve dispersal distance via a certain vector. “If dispersal is advantageous, we would expect to find that diaspores have adaptations that enhance dispersal” (WILLSON & TRAVESET 2000). A determination and classification of the dispersal mode of plant species was, however, until recently more or less **exclusively** done using morphological characteristics of the diaspore (e.g. DANSEREAU & LEMS 1957; LUFTENSTEINER 1982; VAN DER PIJL 1982; GRIME et al. 1988; JENNY 1991). Following these traditional binary classification systems, allocating a species to exactly one mode of dispersal, winged, plumed or balloon-like dispersal units were regarded as anemochorous, those with hooks, awns or bristles as epizoochorous and those with floating diaspores as nautochorous. According to this, endozoochorous dispersal was often restricted to species with fleshy fruits (e.g. LUFTENSTEINER 1982; HUGHES et al. 1994), although numerous feeding experiments were already conducted in the 19th and early 20th century (e.g. KÜHN 1877; KERNER V. MARILAUN 1898; KEMPSKI 1906; KORSMO 1912; HEINTZE 1915), showing that numerous species can survive the passage of the digestive tract of ruminants. Therefore, it might have been accepted since many decades that species with different apparent morphological adaptations are able to survive the passage of the digestive tract of grazing animals as well. However, it often was not the case.

Today, due to more experimental studies during the last decades concerning the dispersal of plant species, it is widely accepted that a strict allocation of a certain diaspore morphology to only one mode of dispersal may lead to wrong interpretations of the real dispersal potential of plant species, because diaspores are usually transported by several dispersal vectors (HOWE & SMALLWOOD 1982; BONN & POSCHLOD 1998a; BONN et al. 2000). Although a simple deduction of the dispersal mode based on certain diaspore traits is misleading, it could also be shown that various characteristics of the diaspore and/or plant are related to the dispersal potential by a certain dispersal vector. Dispersal potential by wind, for example, can be assessed by terminal velocity and release height of the diaspore (ANDERSEN 1991; JONGEJANS & SCHIPPERS 1999; BONN et al. 2000; TACKENBERG 2001; TACKENBERG et al. 2003). Epizoochorous dispersal potential depends on the surface structure of the diaspore and its releasing height (FISCHER et al. 1996). Specific gravity is a decisive diaspore attribute for hydro- (nauto-)chorous dispersal potential (BONN et al. 2000, chap. 3). The knowledge of such determinants for the dispersal potential of plant species by a certain vector has to be regarded as a helpful tool for the risk assessment of plant species (EHRLÉN & VAN GROENENDAEL 1998; POSCHLOD et al. 2000; BONN et al. 2000; POSCHLOD et al. 2004).

Despite the lack of obvious adaptations for endozoochorous dispersal of herbaceous species with non-fleshy fruits there might also exist certain diaspore traits enhancing survival capacity of diaspores. It is, for example, known that hard (= physically dormant) diaspores of legumes are able to survive the passage of a ruminants digestive tract with high rates (e.g. NORTON et al. 1989, DOUCETTE et al. 2001, see chap. 4). Various studies showed relationships between size-related diaspore characteristics and survival capacity (e.g. RUSSI et al. 1992, GHASSALI et al. 1998). The latter relationships were, however, largely restricted to grasses and legumes.

The finding of universally valid plant or diaspore traits correlating with the endozoochorous dispersal capacity of a plant species would render to determine the dispersal potential of a large number of species. A simple deduction of the dispersal potential from – often already existing – data concerning traits of the plant or diaspore would be much easier than a determination of endozoochorous dispersal potential by large-scale feeding experiments or experimental approaches simulating the passage of the digestive tract (chap. 4).

In the present study it was tested whether differences in endozoochorous dispersal potential may be explained by the following diaspore or plant traits:

- It is commonly accepted that the retention time of diaspores in a ruminants gut and its survival rate are negatively correlated (e.g. JANZEN 1984). Therefore, all diaspore traits influencing retention time may also influence the survival rate. Additionally, mastication and rumination act selectively on different feed particles. Diaspore traits favouring the escape of chewing may also effect endozoochorous dispersal potential. In this context, **diaspore size, shape, mass and specific gravity** are discussed as relevant traits (e.g. ATKESON et al. 1934; JANZEN 1984; RUSSI et al. 1992; GARDENER et al. 1993a) and were therefore included in the present analysis.
- All diaspore characteristics offering a defence against the mechanical and chemical attacks during digestion may influence survival capacity during the passage of the digestive tract as well. Anatomical arrangements of tissues, the patterns of lignification and the ease of particle fragmentation during chewing are the major determinants of digestion (KENNEDY & DOYLE 1993). Phenolic compounds in the seed coat contribute to seed hardness, reduce the permeability of the seed coat and built up a barrier against microorganisms (WERKER et al. 1979; MOHAMED-YASSEN et al. 1994; WERKER 1997), because phenols and lignins (hetero-polymers of phenols) are more or less indigestible for ruminal bacteria (VAN SOEST 1994, PALMQUIST 1995). These features were characterized by **the thickness of the embryo and endosperm surrounding structures as well as the content of phenols and lignins**.
- Several diaspore traits favouring a persistent diaspore or seed bank (small size, little mass, spherical shape, resistance of the seed coat against chemical and physical attacks during burial; THOMPSON et al. 1993, 1998; BAKKER et al. 1996, 2000) are also helpful to survive the passage through the digestive tract of ruminants. Consequently, it was tested whether the **seed bank longevity index** (THOMPSON et al. 1998) as an indicator for diaspore persistence in the seed bank is related to endozoochorous dispersal potential.
- Already JANZEN (1984) discussed the existence of evolutionary adaptations of plant species with respect to endozoochorous dispersal and hypothesized that the foliage of herbaceous species “is ecologically the fruit, as well as a photosynthetic machinery…”. If corresponding adaptations do exist, it would be a logical consequence that diaspores of plant species with a high **palatability** survive the passage of the digestive tract better than unpalatable plant species.

- Being dispersed endozoochorously by grazing herbivores first requires the acquisition of diaspores on the pasture. In this context, the **height of diaspore release** and **diaspore supply** play an important role (BONN et al. 2000, chap. 3) and were therefore tested for relations to the number of diaspores in the dung of grazing herbivores.

Methods

Approaches to determine of endozoochorous dispersal potential

Feeding experiment I (IGER, Okehampton)

The feeding experiment was conducted at the IGER Institute in Okehampton, England, with five one year old limousine heifers (mean body weight 300 kg) and five male two year old Texel sheep (mean body weight 60 kg). The animals were fed with a silage of 70% digestibility. In order to guarantee a complete uptake of the diaspores together with the feed, the animals were adapted to bruised grain three days before the beginning of the feeding experiment.

The experiment included 14 plant species of eight plant families (Table 5.1, Fig. 5.1). Species were selected according to the following criteria:

- occurrence on calcareous grasslands (which often have developed as a result of livestock-grazing);
- belonging to different plant families;
- different diaspore traits (diaspore size, morphology, weight, occurrence of hard seeds);
- availability of a large number of diaspores.

6,000 diaspores (commercial seed) of each of the 14 plant species were mixed into bruised grain and fed to each animal. Sheep were separated in single pens during the experiment. The pens were covered with paper shavings, which simplified dung collection after diaspore feeding. Because cattle could not be separated, coloured plastic markers were fed together with the diaspores as well as one day before and two days after diaspore feeding together with the regular feed. The dung of each individual could then be distinguished by the different coloured plastic markers in the excreted faeces. The cattle stable was covered with straw, where a quantitative dung collection proved to be more difficult then in the case of the sheep.

Following the results of previous feeding experiments, which have shown that the retention time of diaspores differs for sheep and cattle (e.g. LENNARTZ 1955; RUSSI et al. 1992; overview in BONN & POSCHLOD 1998a), the timing of dung collection varied between both animal species. Dung collection started 12h after feeding the diaspores to sheep and 17h after feeding to cattle. Faecal material was collected three times per day over a period of eight days (166h) in the case of sheep and nine days (191h) in the case of cattle. During the last three collection days, the dung of only one sheep or cattle was collected, since it was expected that most diaspores were already excreted (e.g. RUSSI et al. 1992; GARDENER et al. 1993a). Each dung sample was weighed and

subsequently dried at room-temperature in order to promote germination (GRIME et al. 1988). 25% of each dung-sample were crumbled, rewetted, mixed with sterile compost and filled in pots, separated after day and time of collection. Faecal material of the last three collection days was exposed to 100%. The samples were exposed in random order in the botanical garden at the University of Marburg (Germany) under natural weather conditions, but wetted if necessary. In order to avoid diaspore input from outside, all pots were covered by a garden fleece. Seedlings were identified and removed in regular time intervals over a period of 15 months, which included a stratification period during winter.

Table 5.1 Plant species, quantities and viability of diaspores fed to cattle and sheep.

plant species	abbrevia-tion	plant family	diaspore origin	diaspores fed/animal	viability before feeding
Achillea millefolium L.	Ach mil	Asteraceae	wild form	6,000	95.9%
Anthyllis vulneraria L.	Ant vul	Fabaceae	wild form	6,000	76.8% (37.8% hard seeds)
Festuca ovina agg.	Fes ovi	Poaceae	cultivated form	6,000	38.2%
Hieracium pilosella L.	Hie pil	Asteraceae	wild form	6,000	65.4%
Inula conyza DC.	Inu con	Asteraceae	wild form	6,000	24.6%
Jasione montana L.	Jas mon	Campanulaceae	wild form	6,000	36.9%
Leucanthemum vulgare LAMK.	Leu vul	Asteraceae	cultivated form	6,000	81.4%
Lotus corniculatus L.	Lot cor	Fabaceae	cultivated form	6,000	87.2% (0.6% hard seeds)
Pimpinella saxifraga L.	Pim sax	Apiaceae	cultivated form	6,000	71.4%
Plantago media L.	Pla med	Plantaginaceae	wild form	6,000	64.2%
Poa angustifolia L.	Poa ang	Poaceae	wild form	6,000	60.0%
Salvia pratensis L.	Sal pra	Lamiaceae	wild form	6,000	61.4%
Sanguisorba minor SCOP.	San min	Rosaceae	wild form	6,000	94.5% (1.6 embryos/ diaspore)
Thymus pulegioides L.	Thy pul	Lamiaceae	wild form	6,000	75,9%

Initial viability of the fed species was determined by germination tests in a climate chamber. 100 to 150 diaspores of each species (5 replications) were placed on two filters in petri dishes. Petri dishes were sealed with parafilm and exposed under a 14h light/22°C, 10h dark/14°C climate-regime. Germinated diaspores were counted and removed twice per week until germination had finished. Ungerminated diaspores were tested for remaining hard (legumes) or firm (grasses, forbs) ungerminated diaspores (ROBERTS 1981). Both germinated and hard or firm ungerminated diaspores were summed up to calculate the viability of a species.

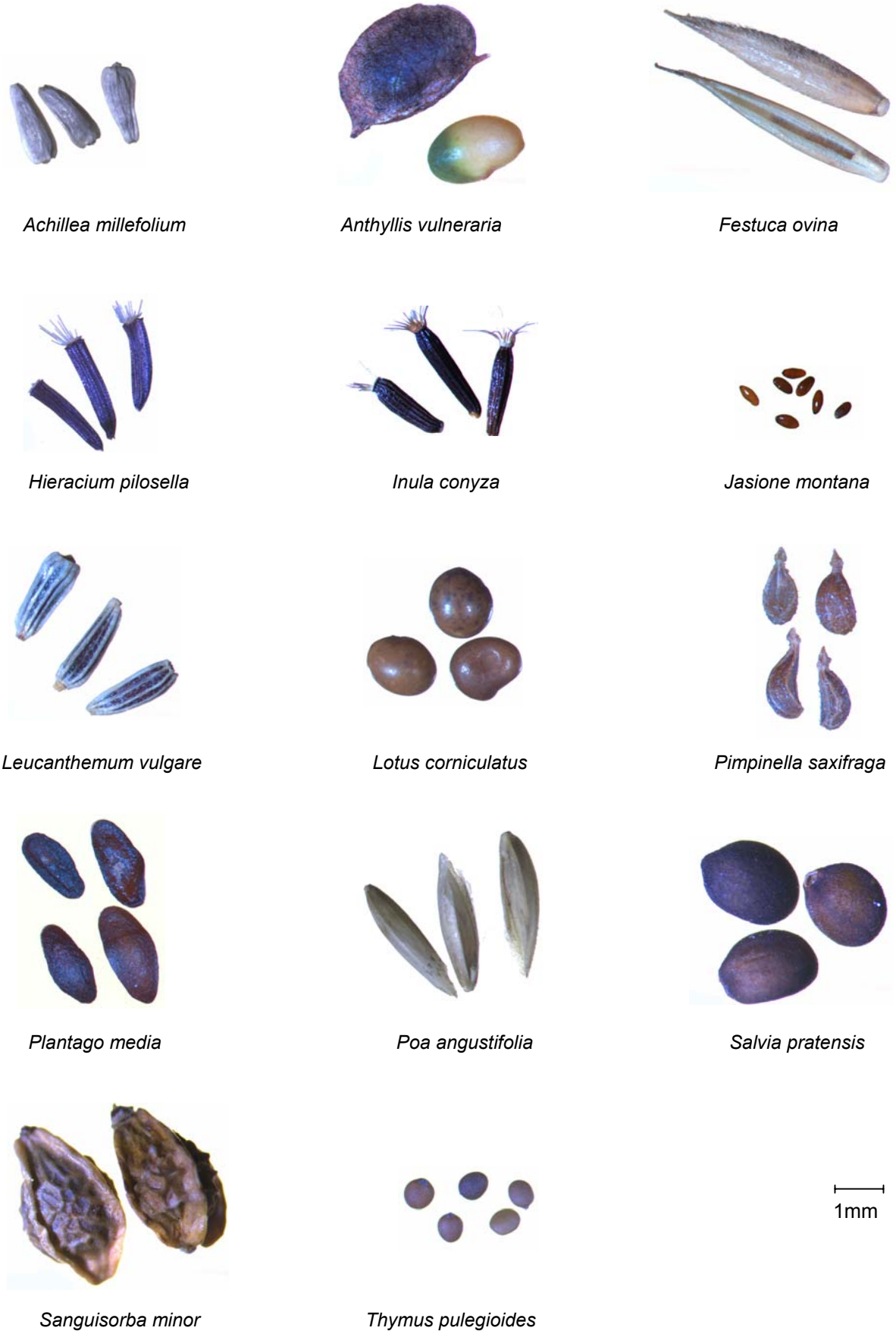


Fig. 5.1 Photographs of the diaspores from the 14 studied species.

Undigested diaspores, exposed under identical conditions as the digested diaspores were used as a control. Therefore, 100 diaspores of each plant species were brought out on cattle faeces mixed with sterilised compost. Cattle dung was collected immediately after voiding and preserved in closed plastic bags at 4°C until the exposure in the botanical garden. These controls were exposed for the same time period as the dung samples from the feeding experiment in the botanical garden. The germination success of fed diaspores in the exposed dung samples was then related to germination of undigested diaspores exposed on dung ("relative germination"). Since it was supposed that all viable diaspores germinated during dung exposure, relative germination of fed diaspores was equated with diaspore survival after passing the digestive tract.

Feeding experiment II (Göttingen)

The experiment was conducted in autumn 1998 in Relliehausen (~50 km northwest of Göttingen, Germany) at the experimental station of the Institute for animal breeding and genetics, University of Göttingen (see LANGHOLZ et al. 2000). Diaspores of 12 plant species (Table 5.2) were either collected in the field or commercially purchased (*Leucathemum vulgare* and *Tragopogon pratensis*). The diaspores were fed to five sheep (Rhoen-sheep) and five cows (Limousin x Holstein). Depending on diaspore availability and expected survival rates (e.g. relatively low survival rates for the Asteraceae), 5,000 to 30,000 diaspores per species were fed to cattle (Table 5.2). Because of their smaller body size, sheep obtained half of the number of diaspores fed to cattle.

Table 5.2 Plant species fed to cattle and sheep in feeding experiment II (Göttingen).

plant species	abbreviation	plant family	no. of diaspores fed		viability before feeding
			cattle	sheep	
***Centaurea jacea** L.*	Cen jac	Asteraceae	15,000	7,500	95.6%
***Daucus carota** L.*	Dau car	Apiaceae	15,000	7500	75.0%
***Holcus lanatus** L.*	Hol lan	Poaceae	15,000	7,500	55.4%
***Leontodon autumnalis** L.*	Leo aut	Asteraceae	30,000	15,000	93.0%
***Leucanthemum vulgare** LAMK.*	Leu vul	Asteraceae	30,000	15,000	76.2%
***Lotus corniculatus** L.*	Lot cor	Fabaceae	15,000	7,500	80.0%
***Medicago lupulina** L.*	Med lup	Fabaceae	12,000	6,000	66.7%
***Pastinaca sativa** L.*	Pas sat	Apiaceae	10,000	5,000	67.0%
***Plantago lanceolata** L.*	Pla lan	Plantaginaceae	15,000	7,500	92.8%
***Salvia pratensis** L.*	Sal pra	Lamiaceae	15,000	7,500	62.1%
***Tragopogon pratensis** L.*	Tra pra	Asteraceae	5,000	2,500	71.6%
***Trifolium pratense** L.*	Tri pra	Fabaceae	15,000	7,500	90.6%

The diaspores of all plant species fed to one animal were mixed and flushed into the reticulo-rumen with water by a gavage. Although initial chewing is prevented by this feeding method, diaspores are subjected to mastication during rumination, which plays an even more important role for particle comminution than initial chewing (ULYATT et al. 1986).

After diaspore feeding, cattle and sheep were allowed to graze on different pastures. Because individuals of each animal species were grazing at the same pasture, a distinction between dung patches of individual animals was not possible. Each pasture was divided into single portions, representing the grazing area for one day. Faecal material of sheep was collected 9 days post feeding, whereas cattle dung was collected 10 days after diaspore feeding. Every day, the animals were driven to the next portion. On the portion grazed the day before, the total number of excreted dung patches was counted. Afterwards 10% of all excreted dung patches were collected, the rest was left on the pasture for further observation.

The collected dung patches were weighed, air dried and crumbled in order to promote germination (GRIME et al. 1988). After rewetting, they were mixed with sterile compost and filled in pots, separated after the day of dung collection. The samples were exposed in an unheated greenhouse, where they were also exposed to a stratification period during winter. Seedlings were identified and removed in regular time intervals until germination had largely finished.

Of half of the dung patches remaining on the pasture size was measured. These dung patches were permanently marked and controlled for seedlings three times after diaspore feeding (six weeks after diaspore feeding, the following spring and summer). Seedlings of the fed species were marked with plastic sticks.

Total survival of each of the fed species was calculated by projecting the number of germinated diaspores to the total number of dung patches excreted every day. If germination rates in the greenhouse and on the pasture differed, the highest seedling number of each species determined either in the greenhouse or on the dung patches left on the pasture was used for further statistical analysis.

Initial viability of the fed species was determined in a climate chamber as described above. Germination rates in the excreted dung were related to diaspore viability determined in the climate chamber and are therefore always given as “relative germination”. Because it was supposed that all viable diaspores germinated during dung exposure, relative germination of fed diaspores was equated with diaspore survival.

Inclusion of feeding experiments from literature

For practical reasons, feeding experiments are usually restricted to a very limited number of species. Consequently, each of the chosen species strongly influences relations between endozoochorous dispersal potential and plant or diaspore traits. It therefore seemed worthwhile to increase the number of species by using data of the two feeding experiments described above as well as data of feeding experiments from literature. Species were included in the analysis when occurring in Central Europe and when the germination capacity or viability of the diaspores before

feeding were comprehensible for the calculation of relative germination after passing the digestive tract. The following experiments were evaluated: KEMPSKI (1906); LENNARTZ (1955); KIRK & COURTNEY (1972); ÖZER & HASIMOGLU (1977); ÖZER (1979); TAKABAYASHI et al. (1979); LACEY et al. (1992); RUSSI et al. (1992); WALLANDER et al. (1995); IDE et al. (1999); DOUCETTE et al. (2001). When the same plant species was fed to a certain animal species in more than one feeding experiment, the results of each experiment were used for statistic analysis.

Dung collections from grazing livestock

Three different studies were evaluated to compare survival-relevant diaspore or plant traits of experimentally fed species with those of species excreted with the dung of animals grazing on pastures (STENDER 1996; KUTZKE 1998; SCHÖNFELDER 1998). All three studies were performed in different habitats grazed by various herbivores or – in one case – omnivores (pigs).

The first study (KUTZKE 1998) was performed in northern Bavaria (Germany) on calcareous grasslands of the Franconian Alb grazed by herded sheep. At each of three dates (29.07.97, 27.08.97, 16.09.97), 30 freshly excreted dung patches were collected. The dung was mixed with sterile compost, filled in pots and cultivated in the greenhouse. Additionally, at each of the three collection dates 120 further dung patches were collected and brought out on three different pastures (40 each). Germinating individuals were regularly evaluated and removed after identification until the beginning of October.

Because species composition and seedling numbers were similar for both cultivation methods (greenhouse and under natural conditions on the pastures), germinated species were summed up for further calculations.

With regard to diaspore supply, phenological data of occurring species were evaluated parallel to dung collection. On each pasture phenology was evaluated by a four-graded scale:

1 fructifying individuals were not registered, but species was germinating in the dung samples (= very low diaspore supply);
2 scattered fructifying individuals, low diaspore supply;
3 moderate diaspore supply;
4 high diaspore supply.

For comparisons of the diaspore supply on a pasture and the diaspore content of excreted dung, a retention time of two days in the digestive tract of sheep was assumed. Therefore, the "dung flora" (i.e. species germinating in the dung of grazing sheep) was compared with the phenological data of the pasture, where sheep grazed two days before. The total diaspore supply was evaluated by summing up the different phenological data of each species.

The second study (STENDER 1996, see also STENDER et al. 1997) was carried out in northern Germany, 50 km east of Bremen. The study area included six moist to wet pastures with up to 111 plant species per pasture, which were grazed since 1990 by Galloway cattle at a low intensity.

In order to determine the content of viable diaspores in the faeces of the Galloway cattle, two dung patches were collected on each of the six grazed pastures every 14 days beginning at the 09.06.95 until the end of September. On pastures where the grazing period started later or ended earlier, dung collection was modified correspondingly. In total, 48 dung patches were collected. The patches were collected except for the lowest layer in order to avoid contamination with soil or diaspores lying on the ground.

After weighing each dung sample was mixed with sterile compost, filled in pots and dried for two weeks in order to promote germination (GRIME et al. 1988). After rewetting, the samples were cultivated in a greenhouse. Seedlings were regularly determined and removed. In order to stimulate germination of dormant species, cultivation was interrupted by a stratification period (2°C) of six weeks in a climate chamber. The cultivation of the dung samples ended in May 1996.

Abundance of fructifying plant species on the different pastures was estimated during the vegetation period in four classes:

1 rare (1-5 individuals per pasture);
2 scattered (< 1 induvual/m²);
3 frequent (> 1 indivual/m²);
4 dominant.

The third study (SCHÖNFELDER 1998) was performed on flood-plain meadows of the river Sava (Crotia), grazed by cattle, horses and pigs.

Pig dung was collected every two weeks from the beginning of May 1997 until the beginning of September 1997. At each date, 16 freshly excreted dung samples were collected. The dung samples were weighed, mixed with sterilized compost, filled in pots and covered with a fleece. The samples were cultivated outdoors, but provided with a continuous water supply. Cultivated dung samples were regularly controlled for seedlings, which were removed after determination. After a frost period sufficient for stratification of dormant diaspores, half of the dung samples were cultivated in a greenhouse until the end of April 1998.

Cattle and horse dung was collected at only three dates (end of June, mid and end of July). Of each animal species, eight freshly deposited dung samples were collected per date. The patches were collected completely except for the lowest layer in order to avoid a contamination with soil or diaspores lying on the ground. The cultivation of the samples was the same as described for pig dung.

The quantity of fructifying individuals per plant species was estimated every two weeks during the period of dung collection using a five graded scale:

1 rare (< 1 individual/10m²);
2 scattered (< 1 individual/m²);
3 regularly occurring (1-10 individuals/m²);
4 frequent (> 11-50 individuals/m²);
5 very frequent (> 50 individuals/m²).

Additionally, duration of fructification was estimated on each date by a four graded scale:

1 < two weeks/not registered but species germinated in dung;
2 +/- two weeks;
3 +/- four weeks;
4 +/- six weeks.

Total diaspore supply per species was calculated by multiplying the mean quantity of fructifying individuals with the duration of fructification.

In each study, the total number of diaspores germinated in the collected dung was equated with 100%. The "relative frequency" of germinating diaspores of each plant species in the collected dung relates to the total number of germinated diaspores.

Evaluation of diaspore and plant traits

Diaspore traits

Morphological traits of the fed species (diaspore length, width, height) as well as diaspore mass were measured for 10 diaspores of each species fed in feeding experiment I and II. Diaspore shape was calculated after THOMPSON et al. (1993). All Poaceae were measured with glumes. Except for *Anthyllis vulneraria,* all legume-diaspores were measured without pods, because they were removed before feeding. Diaspore traits of the species fed in various feeding experiments from literature were either taken from different data bases (BONN et al. 2000; KLOTZ et al. 2002), literature or measured on commercial seed as described above. Diaspore traits of species found in the dung samples of grazing herbivores or on the grazed pastures were exclusively taken from data bases (BONN et al. 2000; KLOTZ et al. 2002). For grasses, diaspore size with glumes was used for the analysis, for all other species measures without easy detachable appendages were chosen.

Specific gravity of the diaspores was determined via different concentrations of ethanol and saccharose solutions from 0.8 to 1.3 g/ml in 0.05 g/ml steps. Specific gravity could not be evaluated for the species found in the dung samples of grazing herbivores and in pasture vegetation, because a determination was not possible for the numerous species.

The seed bank longevity index (BEKKER et al 1998; THOMPSON et al. 1998) was calculated using seed bank persistence from the database of THOMPSON et al. (1997), supplemented with data from POSCHLOD et al. (1991); GRUNICKE (1996); BIEDERMANN (1998); KIEFER (1998) and JACKEL (1999).

Plant traits

Release height, which only was related to the frequency of plant species in dung samples from grazing herbivores, was taken from the database "Diasporus" (BONN et al. 2000).

Data concerning plant species palatability were taken from the database BIOFLOR (KLOTZ et al. 2002), in which palatability is differentiated by a nine-graded scale according to ELLENBERG's indicator values (BRIEMLE et al. 2002, see also NITSCHE 1993). Although these values actually indicate the forage value of plant species (see also KLAPP et al. 1953), where palatability is only one of several criteria for the valuation of plant species, they also represent a qualified estimation of feed preferences of livestock (Prof. Dr. JAKOB, Hohenheim, personal communication).

Characteristics of the "seed coat"

Characteristics of the "seed coat" were measured only for the plant species fed in feeding experiment I (Table 5.1). For measurements of the "seed coat", it was assumed that all structures surrounding the embryo and endosperm may prevent the embryo from chemical or microbial attacks during digestion and are therefore important for the potential to survive the passage of the digestion tract, regardless if they belong to the intrinsic seed or fruit coat in the morphological sense. In the following, the term "seed coat" will be used for these surrounding structures.

Measurements of the thickness of the seed coat, its content of phenols and lignins were done by microtome sections. Diaspores without glumes and pods were put in Eppendorf cups (0.5 ml) and imbibed for 30 minutes at room temperature (RT) in a phosphate buffer with pH 7.4 after Sörensen. Fixation of the tissue took place in 5% (v/v) glutaraldehyde in phosphate buffer after Sörensen for 24h at 4°C. Big diaspores were broached with a thin needle before imbibition and fixation to improve penetration of the fluids. After fixation the diaspores were dehydrated in a graded ethanol series (30%, 50%, 70%, 80%, 95%, 99,5%, 100% Ethanol, 10 min each at RT). From 95% Ethanol on, the dehydration was done under vacuum in an excsicator. The following pre-infiltration was done with an Ethanol (100%)-Technovit 7100 (Heraeus-Kulzer, Wehrheim, Germany) solution (1:1 v/v) under vacuum for 2h at RT. For infiltration the solution was exchanged by a mixture of Technovit 7100 (100ml) and a hardener (1 g Dibenzoylperoxid) for 16h at RT. The infiltration solution was changed once during infiltration. Finally, infiltration solution and a second hardener were mixed 15:1 for embedding (2h at RT).

Sections of 3 µm thickness were obtained by a microtome (Autocut, Reichert & Jung, Microsystems, Germany) equipped with a glass knife. Sections were attached to glass slides and stained with a mixed safranin-astrablue-solution. These dyes stain phenolic or lignified cell walls red, weaker lignified parts pink and non-lignified parts blue (GERLACH 1977, BRAUNE et al. 1991). Astrablue- and safranin-solutions were mixed in a ratio 40:1 (v/v) (BRAUNE et al. 1991). Sections were stained between two and four minutes and then washed with H_2O dest.

For microscope observation and photographs, a Zeiss Microscope (Axiomat) and a digital microscope camera (AxioCam, Carl Zeiss, Germany) were used. The images served for measurements of the following diaspore traits:

a. total thickness of the seed coat (including cell lumen, "brutto thickness") (see Fig. 5.2: a.);

b. thickness of the largest phenol-containing (red stained) layer (Fig. 5.2: b). When the biggest phenol containing layer was stained pink instead of red (*Leucanthemum vulgare, Plantago*

media, Salvia pratensis (Fig. 5.2b), *Sanguisorba minor*), measured thickness was halved, because a pink instead of a red coloration indicates a weaker lignification (GERLACH 1977);

c. thickness of the seed coat without cell lumen ("netto thickness");

d. total thickness of all phenol-containing (red stained) cell-layers without cell lumen.

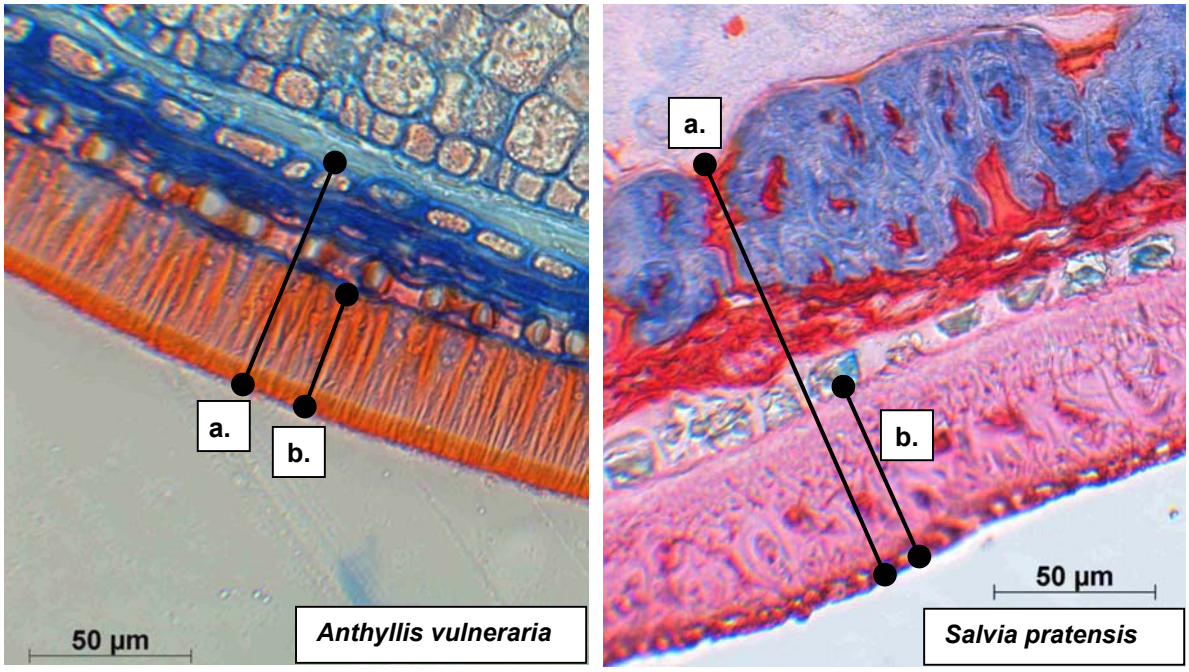


Fig. 5.2 Measurements of the "seed coat" on diaspore sections stained with safranin-astrablue. a.: total thickness of the "seed coat", b.: thickness of the largest phenol-containing layer.

Measurements were performed using an automatic image analyser (KS300 Imaging System, Vers. 3.0, Carl Zeiss, Germany). Of each of the 14 species five diaspores (five sections each) were analysed by five measurements per section. Total thickness of the seed coat (Fig. 5.2: a) as well as thickness of the biggest red coloured layer (Fig. 5.2: b) were directly measured, choosing a thinner part of the surrounding structures – as the most sensitive parts – for measurements. "Netto thickness" (thickness of the seed coat without cell lumen, c.) and total share of phenol containing layers of the seed coat (d.) were calculated by KS300 using macros. These macros partitioned the different colours of an image into a binary picture and measured the number of pixels of one of the two colours. The resulting percentage was referred to the measured total thickness of the seed coat.

Data analysis

Data analysis were performed with relative germination of diaspores fed in the different feeding experiments and relative germination of fructifying species of each pasture in relation to the total species number germinated in the collected dung samples.

Relations between relative germination of diaspores in the dung samples and different plant or diaspore traits were analysed using Spearman's rank correlation. The analysed diaspore and plant attributes and site-specific variables, respectively, differed among the different studies (Table 5.3).

Table 5.3 Variables evaluated for the different studies.

	feeding experiment I (IGER)	feeding experiment II (Göttingen)	incl. feeding experiments from literature	dung collected from grazing herbivores
diaspore length	■	■	■	■
diaspore width	■	■	■	■
diaspore height	■	■	■	■
diaspore shape	■	■	■	■
diaspore mass	■	■	■	■
specific gravity	■	■	■	
seed bank longevity index	■	■	■	■
species' palatability	■	■	■	■
total thickness of the seed coat (a.)	■			
thickness of the biggest phenol-containing (b.)	■			
thickness of the seed coat without cell lumen (c.)	■			
total thickness of all phenol-containing layers (d.)	■			
diaspore supply				■
release height				■

Stepwise discriminant analyses were performed to identify attributes of the diaspore, plant or site (pasture) (Table 5.3) assumed as suitable to predict endozoochorous dispersal potential. In order to do this, survival rates of plant species had to be classified. Although a more detailed classification would be desirable (chap. 4), the limited number of species fed in feeding experiment I and II only allowed a distinction of two groups. Germination rates of the aggregated feeding experiments as well as those from dung samples of grazing herbivores collected on the pasture

could be divided into three groups. The classes differed between the single approaches (Table 5.4):

- for the feeding experiments, the lowest class (none surviving species) included also species with low survival rates (≤ 0.5% relative germination), because diaspores of those species were mostly excreted only by single animals despite the, in relation to natural conditions, high diaspore numbers fed.
- maximum proportions of germinating species in the dung of grazing animals were usually much lower than relative germination rates of the best surviving species in the evaluated feeding experiments. Therefore, different limits for each class had to be chosen (Table 5.4).

Table 5.4 Classifications of relative germination of fed diaspores and relative germination in dung collected from grazing herbivores, respectively, for discriminant analysis.

	feeding experiment 1 (IGER)	**feeding experiment 2 (Göttingen)**	**incl. feeding experiments from literature**	**dung collected from grazing livestock**
two groups	≤0.5%; >0.5%	≤0.5%; >0.5%	≤0.5%; >0.5%	0%; >0%
three groups			≤0.5%; >0.5-5%; >5%	0%; >0-1%, >1%

Discriminant analyses were performed for the following trait-combinations:

1) all variables evaluated for the different studies (Table 5.3);
2) all measured diaspore traits (diaspore size, mass, specific gravity as well as seed coat characteristics);
3) seed bank longevity index and palatability as well as release height and diaspore supply, if evaluated.

All statistical analysis were done using SPSS (Vers. 11.0).

Results

Feeding experiment I (IGER, Okehampton)

Survival of plant species fed to sheep and cattle

With a relative germination between 16.3% and 34.2%, *Anthyllis vulneraria* and *Achillea millefolium* survived the passage of the digestive tract of both ruminant species best (Fig. 5.3). Significant differences in diaspore survival ($p<0.05$) after passing the alimentary tract of cattle or sheep could be found for five species: *Plantago media, Thymus pulegioides*, *Hieracium pilosella* and *Salvia pratensis* survived the passage of the sheep gut in higher quantities, whereas *Sanguisorba minor*

survived the passage of the cattle digestive tract better (chap. 4: Table 4.6). *Jasione montana* and *Inula conyza,* which had already very low germination rates when sown on dung, germinated neither in sheep nor in cattle dung after passing the alimentary tract.*

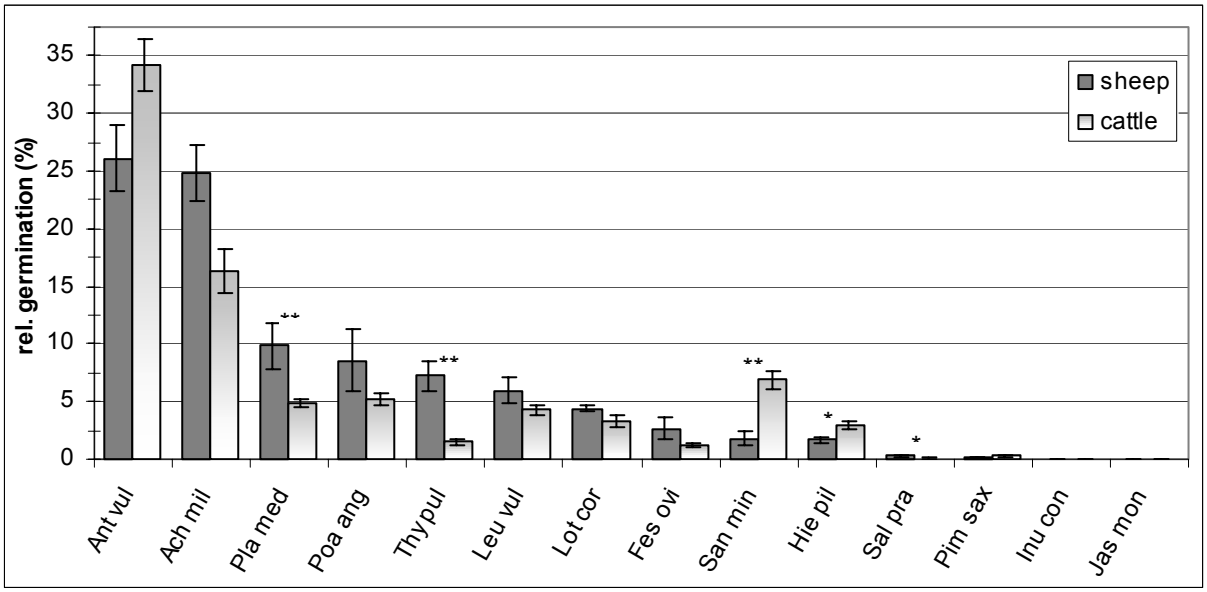


Fig. 5.3 Relative germination of 14 plant species fed to cattle and sheep (in relation to undigested diaspores exposed under the same conditions than digested ones).

Abbreviations for plant species see Table 5.1; bars: ± SE; tests for differences in relative survival between sheep and cattle were done separately for each plant species by U-Test; ** = p<0.01; * = p<0.05.

Diaspore traits of the fed plant species

Measured diaspore traits are documented exemplary for the species of feeding experiment I. Between the smallest and the largest diaspores a nine-fold range in length (0.54 to 4.84 mm, Table 5.5), a seven-fold range in width (0.27 to 3.33 mm) and a 15-fold range in diaspore height (0.19 to 2.88 mm) was observed. A similar broad range existed in the variance of diaspore dimensions (0.01 to 0.15), whereas diaspores differed most in seed weight (0.02 to 10.42 mg). Specific gravity varied from 0.78 to 1.03 g/cm^3 (Table 5.5), the seed bank longevity index (THOMPSON et al. 1998) from 0 to 1.

Characteristics of the "seed coat" varied greatly among species (Table 5.6). The fruit of *Sanguisorba minor* proved to have the most extensive seed coat as well as the biggest phenol-containing layers. However, thickness of the biggest phenol-containing layer was somewhat higher in *Anthyllis vulneraria* (Fig. 5.2). In the Asteraceae, *Leucanthemum vulgare* proved to have the biggest magnitude of surrounding structures, which were, however, quite porous. Its thickest

* For further details see chapter 4.

phenol layer was even thinner than the one of *Achillea millefolium* (Fig. 5.9), which had very few protecting structures. The same accounts even more for *Plantago lanceolata* (Fig. 5.9) and both Poaceae. The diaspore of *Plantago lanceolata* was characterized by very thin, porous surrounding cell layers. In the case of *Festuca ovina* and *Poa angustifolia* (Fig. 5.9) especially the region around the embryo was only covered by thin protective structures.

Table 5.5 Mean values of the evaluated diaspore traits of species fed to cattle and sheep in feeding experiment I.

Abbreviations for plant species: see Table 5.1.

	length (mm)	width (mm)	height (mm)	shape (0 ≤ 0.2)	mass (mg)	specif. gravity (g/cm^3)	longevity index
Ach mil	1.89	0.66	0.26	0.13	0.15	0.83	0.13
Ant vul	3.95	2.55	1.36	0.07	3.18	0.88	0.20
Fes ovi	4.79	0.96	0.68	0.15	0.79	0.83	0.10
Hie pil	2.31	0.42	0.40	0.15	0.17	0.83	0.29
Inu con	1.92	0.47	0.39	0.13	0.23	0.83	1.00
Jas mon	0.54	0.27	0.19	0.08	0.02	0.78	0.50
Leu vul	2.36	0.82	0.63	0.11	0.47	0.88	0.46
Lot cor	1.44	1.28	1.16	0.01	1.33	1.03	0.27
Pim sax	1.69	0.87	0.60	0.08	0.42	0.93	0.19
Plan med	2.04	1.01	0.44	0.11	0.39	0.93	0.38
Poa ang	2.95	0.71	0.69	0.13	0.31	0.83	0.40
Sal pra	1.90	1.67	1.35	0.02	1.82	0.88	0.00
San min	4.84	3.33	2.88	0.03	10.42	0.93	0.46
Thy pul	0.73	0.67	0.53	0.01	0.14	0.93	0.55

Correlations of diaspore and plant traits with survival rates after passing the digestive tract of ruminants

There was no significant correlation between relative germination of fed diaspores after passing ruminants guts and any of the chosen plant and diaspore traits. The best correlation with relative germination after passing digestive tract of cattle was obtained for diaspore length (r=0.50, p=0.06, Spearman's rank correlation coefficient), whereas survival after passing the digestive tract of sheep showed no correlation approximating significance.

Table 5.6 Mean values of the evaluated traits of the "seed coat" * of diaspores fed to cattle and sheep in feeding experiment I.

[1]: measured thickness divided by 2, because of pink coloration (see methods for details).

	total thickness of the seed coat (µm)	total thickness of the seed coat without cell lumen (µm)	thickness of the biggest phenol-containing (red) layer (µm)	total thickness of phenol-containing (red) cell-layers (µm)
Achillea millefolium	17.2	14.7	9.0	13.9
Anthyllis vulneraria	82.9	75.4	40.8	46.9
Festuca ovina	9.7	8.5	4.5	7.8
Hieracium pilosella	20.3	18.3	18.3	14.7
Inula conyza	20.2	18.1	19.7	17.1
Jasione montana	14.1	13.5	11.7	11.6
Leucanthemum vul.	75.4	58.4	4.1[1]	19.6
Lotus corniculatus	83.1	73.7	34.8	35.8
Pimpinella saxifraga	19.0	16.8	14.6	13.1
Plantago media	17.7	6.1	2.2	4.2
Poa angustifolia	9.8	8.7	6.9	6.1
Salvia pratensis	133.5	112.7	26.9[1]	58.0
Sanguisorba minor	192.4	148.9	33.8[1]	67.3
Thymus pulegioides	34.7	33.3	22.8	24.3

Discrimination of grouped data of diaspore survival by diaspore and plant traits

No variable was found to discriminate among any of the different groups.

Feeding experiment II (Göttingen)

Survival of plant species fed to sheep and cattle

After passing the alimentary tract of sheep and cattle germination rates of diaspores were drastically reduced in relation to diaspore viability determined in the climate chamber (Fig. 5.4). In contrast to feeding experiment I (IGER) (Fig. 5.3), most species showed higher relative germination rates in cattle dung. Comparing relative germination of the three plant species fed in both experiments (*Leucanthemum vulgare, Lotus corniculatus, Salvia pratensis*), relative germination of *Lotus* was markedly higher in feeding experiment II. However, with an initial hard seed content of

* It was assumed that all structures surrounding the embryo and endosperm may prevent the embryo from chemical or microbial attacks during digestion and are therefore important for the potential to survive the passage of the digestion tract, regardless if they belong to the intrinsic seed or fruit coat in the morphological sense. Therefore, the term "seed coat" is used for these surrounding structures.

5.8%, the number of hard seeds was about 10 times higher than in feeding experiment I (0.6% hard seeds). In contrast, *Leucanthemum vulgare* was one of the worst surviving species in experiment II (Göttingen) (no seedlings in cattle dung, 0.8% relative germination in sheep dung), whereas it showed a relative germination between 2.0 and 2.7% in experiment I. *Salvia pratensis* survived the passage of the digestive tract in both experiments only in very low quantities.

Most species showed higher germination rates in the greenhouse than on the dung patches left on the pasture. However, the three legumes fed germinated in most cases in higher quantities on the pasture. The same accounts for diaspores of *Tragopogon pratensis* as well as for *Daucus carota* and *Salvia pratensis* excreted by sheep.

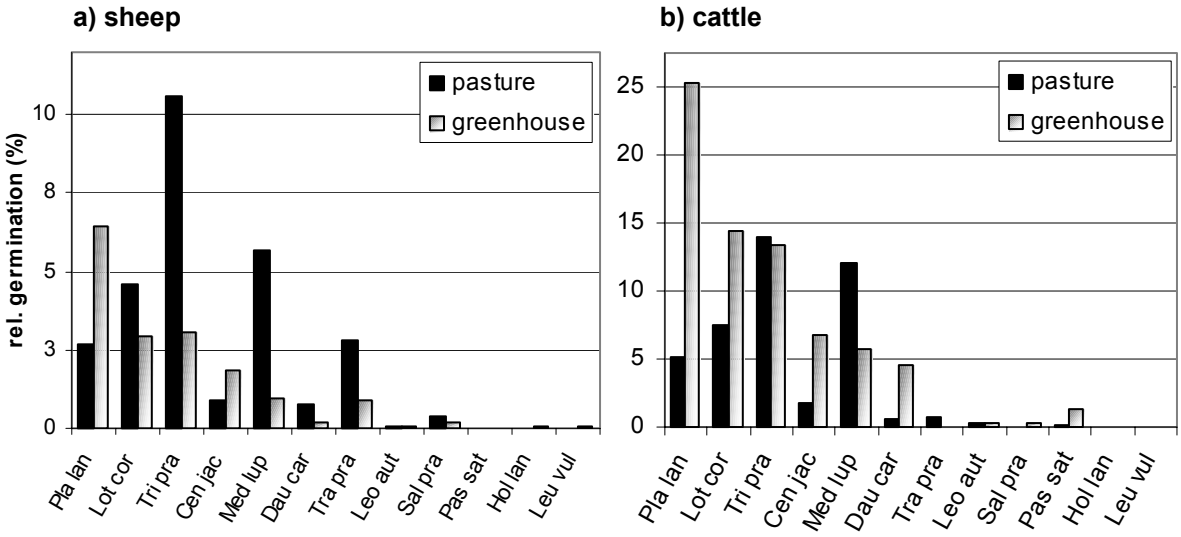


Fig. 5.4 Relative germination of diaspores fed to sheep (left) and cattle (right), with the dung samples exposed in the greenhouse or left on the pasture in relation to diaspore viability determined in the climate chamber.

Abbreviations for plant species see Table 5.2.

Correlations of diaspore and plant traits with survival rates after passing the digestive tract of ruminants

Similar significant correlations between diaspore or plant species' traits and survival rates (relative germination in dung) after passing the digestive tract were obtained for cattle and sheep. Relative germination of the plant species fed proved to correlate significantly with three of the diaspore or plant traits evaluated: diaspore shape, specific gravity and plant species' palatability. Diaspore shape was correlated negatively with survival of the fed species, whereas specific gravity as well as species palatability showed positive correlations (Fig. 5.5, Table 5.10).

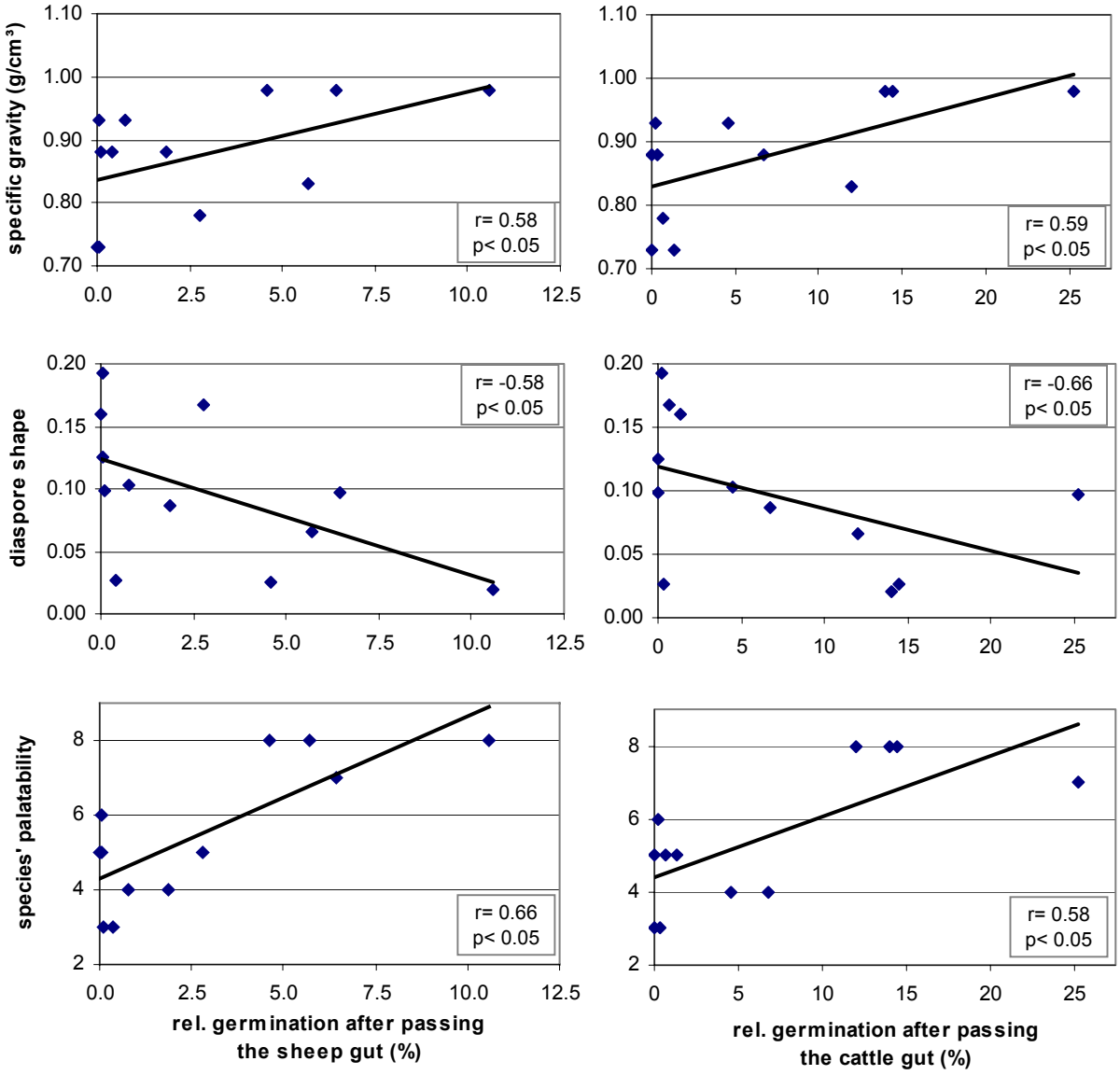


Fig. 5.5 Correlations between survival of diaspores fed to ruminants (sheep (left column) and cattle (right column)) and diaspore shape, specific gravity and species' palatability.
Spearman's rank correlation coefficient, n=12.

Discrimination of grouped data of diaspore survival by diaspore and plant traits

Discriminant analyses reflected the results of the found correlations only poorly (Table 5.7). When data were grouped in surviving and non- or only insignificantly (≤0.5%) surviving species the discriminant analysis did not identify a significant model by the chosen variables for survival rates obtained after passing the sheep gut. For cattle, diaspore mass and species' palatability discriminated the two groups when all explaining variables were included in the analysis (Table 5.7: 1). According to the standardized canonical discriminant function, the coefficient of plant species' palatability had a higher relative importance than diaspore mass. With a Wilks's lambda

factor of 0.39 at p<0.01 and a classification-accuracy of 91.7% the discrimination with the two variables showed a high reliability.

When the explaining variables were restricted to diaspore traits concerning morphology and mass (2), only diaspore mass was selected for discrimination. However, the result of the discrimination was less reliable. No model could be developed when only seed bank longevity index and species' palatability were used (3).

Table 5.7 Results of stepwise discriminant analyses for data grouped with regard to the survival of diaspores of 12 plant species after passing the digestive tract of sheep and cattle (two groups: survival ≤0.5% or >0.5%).

1) all variables included (measured diaspore traits, seed bank longevity index and palatability; s. Table 5.3)
2) only measured diaspore traits included
3) only longevity and palatability included

*: significant at p<0.05; **: significant at p<0.01.

animal:	sheep	cattle
discriminating variables; standardized canonical discriminant function coefficients in brackets	-	1) diaspore mass (0.962), palatability (0.796) 2) diaspore mass (1) 3) -
	quality of discrimination:	
eigenvalue	-	1) 1.600 2) 0.722 3) -
Wilks's λ	-	1) 0.385 ** 2) 0.581 * 3) -
correctly classified	-	1) 91.7% 2) 75% 3) -

Inclusion of results of various feeding experiments from literature

By combining data of various published feeding experiments with the results of feeding experiment I and II (IGER and Göttingen), 63 data sets of 57 species fed to sheep and 64 data sets of 53 species fed to cattle were obtained, including grassland as well as ruderal and weedy species. Species fed to sheep included 6 Poaceae and 11 legumes, those fed to cattle 15 Poaceae and 5 legumes.

Data concerning morphological diaspore traits, diaspore mass and specific gravity were available for all species fed to cattle whereas specific gravity could not be evaluated for one species fed to sheep. Longevity index could be evaluated for all but two species fed to sheep and

for all but one fed to cattle. Data concerning species' palatability were available for 49 species (=55 data sets) fed to sheep and 45 species (=56 data sets) fed to cattle.

Correlations of diaspore and plant traits with survival rates after passing the digestive tract of ruminants

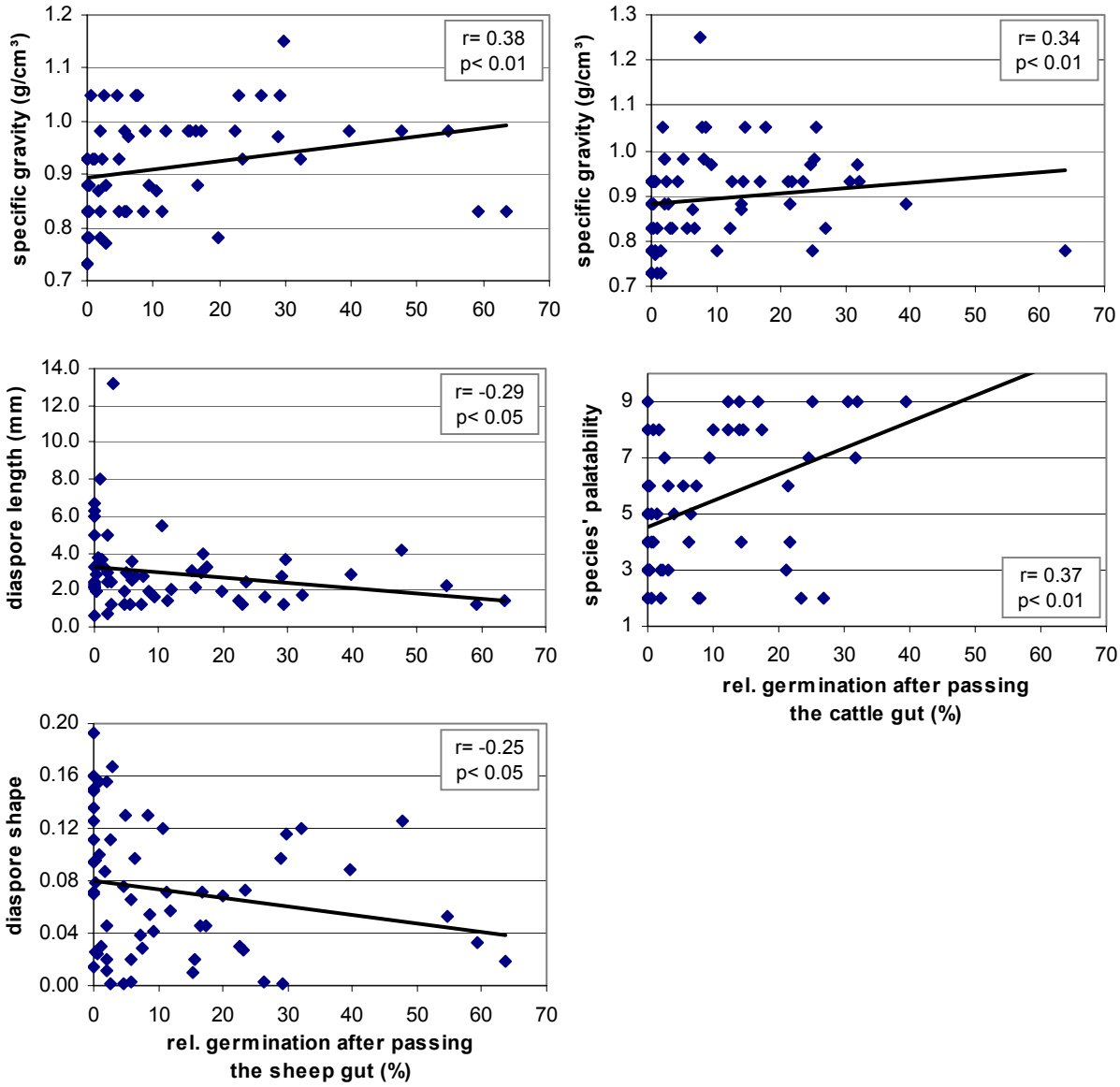


Fig. 5.6 Correlations between survival of diaspores fed to ruminants (sheep (left column) and cattle (right column)) and diaspore length (sheep: n=63; cattle: n=64), shape (sheep: n=63; cattle: n=64), specific gravity (sheep: n=62, cattle: n=64) and species' palatability (sheep: n=55; cattle: n=56).

Spearman's rank correlation coefficient.

Diaspore survival after passing the alimentary tract of sheep was significantly correlated with diaspore length and shape. Specific gravity proved to be positively correlated with relative survival of plant species after passing the alimentary tract of both ruminants (Fig 5.6, Table 5.10). Species recovered from the alimentary tract of cattle showed significant correlations with specific gravity and plant species' palatability. However, with correlation coefficients between 0.25 and 0.37 all these correlations were quite low.

Discrimination of grouped data of diaspore survival by diaspore and plant traits

For a two-group classification, grouping data into non or only insignificantly surviving species (≤0.5%) and those surviving the passage of the digestive tract of sheep in higher quantities (>0.5%), the two groups were discriminated by specific gravity, diaspore mass and diaspore height, when all independent variables were included in the analysis (Table 5.8, 1). The same result was obtained, when only morphological parameters, diaspore mass and specific gravity were used as explaining variables (2). The reduction of the number of explaining variables improved classification accuracy from 64.3 to 72.6%, although both eigenvalues decreased when the number of explaining variables was reduced. No result was obtained, when only seed bank longevity index and plant species' palatability were used as explaining variables (3).

Using all independent variables to discriminate diaspore survival after passing cattle gut (1) for a two-group classification, specific gravity, seed bank longevity index and species' palatability were chosen as discriminating variables. Again, a low eigenvalue indicates a poor separation of the two groups, but 71.0% of all cases were classified correctly. When the number of explaining variables was reduced (2, 3), the reliability of the discriminant function decreased.

Separating relative survival of plant species into three groups did not improve the discrimination of the data in any case (Table 5.8). Survival rates after passing the digestive tract of sheep were discriminated by specific gravity, when the analysis was conducted with morphological diaspore traits, diaspore mass and specific gravity (2). However, classification accuracy amounted to only 56.5%.

Plant species' palatability and diaspore length discriminated grouped recovery rates from cattle, when all explaining variables were included in the analysis (1). Again, only slightly more than 50% of all data were correctly classified. When the number of explaining variables was reduced, correctness of data classification remained constant or even decreased (2, 3).

Table 5.8 Results of stepwise discriminant analyses for data grouped with regard to the survival of diaspores of different plant species after passing the digestive tract of sheep and cattle.

1) all variables included (measured diaspore traits, seed bank longevity index and species palatability; see also Table 5.3)
2) only measured diaspore traits included
3) only longevity and palatability included

Figures separated by "/" relate to the different functions of multiple discriminant analysis; *: significant at p<0.05; **: significant at p<0.01; ***: significant at p<0.001.

	TWO GROUPS (0≤0.5%; >0.5%)		**THREE GROUPS** (≤0.5 %; ≤5%; >5%)	
	sheep	**cattle**	**sheep**	**cattle**
discriminating variables; standardized canonical discriminant function coefficients in brackets	1) specific gravity (1.008), diaspore mass (-1.278), diaspore height (0.763) 2) specific gravity (0.985), diaspore mass (-1.209), diaspore height (0.789) 3) -	1) specific gravity (0.593), longevity index (0.663), palatability (0.819) 2) specific gravity (1) 3) longevity index (0.774), palatability (1.016)	1) - 2) specific gravity (1) 3) -	1) palatability (1.115 / 0.338), length (-0.862 / 0.783) 2) specific gravity (1) 3) palatability (1)
	quality of discrimination:			
eigenvalue	1) 0.407 2) 0.342 3) -	1) 0.291 2) 0.068 3) 0.190	1) - 2) 0.160 3) -	1) 0.346 / 0.017 2) 0.142 3) 0.166
Wilks's λ	1) 0.711 *** 2) 0.745 *** 3) -	1) 0.774 ** 2) 0.936 * 3) 0.841 *	1) - 2) 0.862 * 3) -	1) 0.730 *** 2) 0.876 * 3) 0.858 *
correctly classified	1) 64.3% 2) 72.6% 3) -	1) 71.0% 2) 56.3% 3) 66.1%	1) - 2) 56.5% 3) -	1) 55.4% 2) 43.8% 3) 55.4%

Dung collections from grazing livestock

Species germinating in the dung excreted by different grazing herbivores

Between 38% and 57% of all fructifying plant species documented in the different grazed pastures were found in the collected dung samples. The lowest species numbers germinated in the dung of horses and pigs (38% or 23 species each), whereas 35 of in total 61 fructifying species (57%) germinated in cattle dung of the same study (SCHÖNFELDER 1998). In cattle dung collected by STENDER (1997) 43% of all 147 fructifying species germinated in the exposed dung samples. About half (51%) of the 74 species fructifying on the pasture during grazing germinated in the dung excreted by grazing sheep (KUTZKE 1998).

Data concerning morphological diaspore traits were available for all species, whereas diaspore mass was missing for two species of study 3 (SCHÖNFELDER 1998). Seed bank longevity

index could be evaluated for 97% of all species of study 1 (KUTZKE 1998), 72% of study 2 (STENDER 1996) and 92% of study 3 (SCHÖNFELDER 1998). Data concerning plant species' palatability were available for 97% (study 1), 87% (study 2) and 92% (study 3) of all species, respectively.

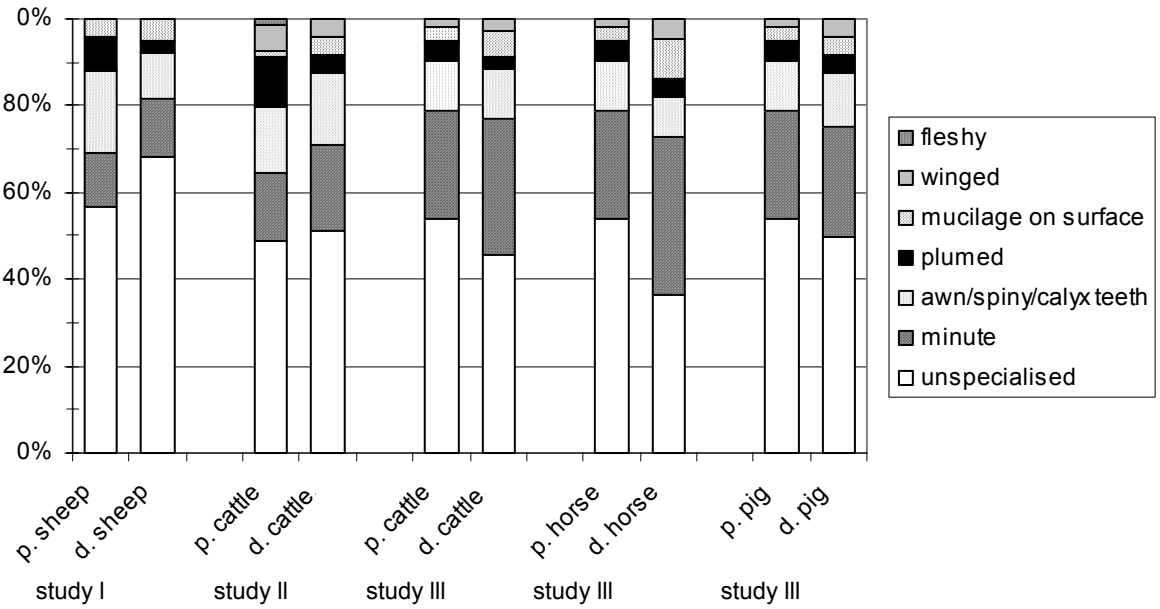


Fig. 5.7 Relative proportion of morphological diaspore types fructifying on the pasture (p.) and germinating in collected dung samples (d.).
p. sheep: pasture sheep, d. sheep: dung sheep, p. cattle: pasture cattle, d. cattle: dung cattle etc.

Comparing the spectra of morphological diaspore types fructifying in the grazed vegetation and germinating in the exposed dung samples, only minute diaspores had in all studies similar or higher relative frequencies in the collected dung samples than on the pasture (Fig. 5.7). The proportion of species with diaspores lacking any obvious adaptation for dispersal in space, which were most frequent on all pastures, was variable between the pasture and dung. The same is true for diaspores with awns, spiny ones and such with calyx teeth. All other morphological diaspore types were rare on the pasture and germinated – except of winged diaspores – less frequent in the collected dung samples.

Correlations of traits of the diaspore, plant and site (pasture) with diaspore frequency in the dung of grazing herbivores

Each of the explaining variables included in the correlation analysis (Spearman's rank correlation; Table 5.3) showed significant correlations with relative germination of plant species in the dung of different herbivores in at least one study (including species fructifying but not present in the dung samples = 0% relative germination) (Fig. 5.8a-e).

Significant negative correlations between at least two of the evaluated morphological diaspore traits (length, width, height, shape) and the relative frequency of fructifying plant species in the collected dung samples were obtained for all studies except for the one examining the dung of pigs (SCHÖNFELDER 1998). However, all correlations were very low (r< 0.35; Fig. 5.8a-d). Additionally, diaspore mass was negatively correlated with the relative frequency of plant species in the dung of cattle (STENDER 1996) and horse (SCHÖNFELDER 1998). Germination rates of fructifying plant species evaluated in these two studies showed also negative correlations with the seed bank longevity index (Fig. 5.8b, d). Except for one study, height of diaspore release correlated negatively with plant species' relative frequency in dung. With the exception of germination rates obtained in the collected cattle dung (SCHÖNFELDER 1998) these correlations were highly significant. Plant species' palatability was in only one study significantly correlated with relative frequency of fructifying plant species in the collected dung samples (STENDER 1996).

Except for the species germinating in the dung of sheep, the highest correlation coefficients were obtained when correlating relative frequency in the dung samples with phenological data. Total diaspore supply during the grazing period was correlated highly significant with the relative frequency of plant species in the dung of cattle, horse and pig (Fig. 5.8b-e).

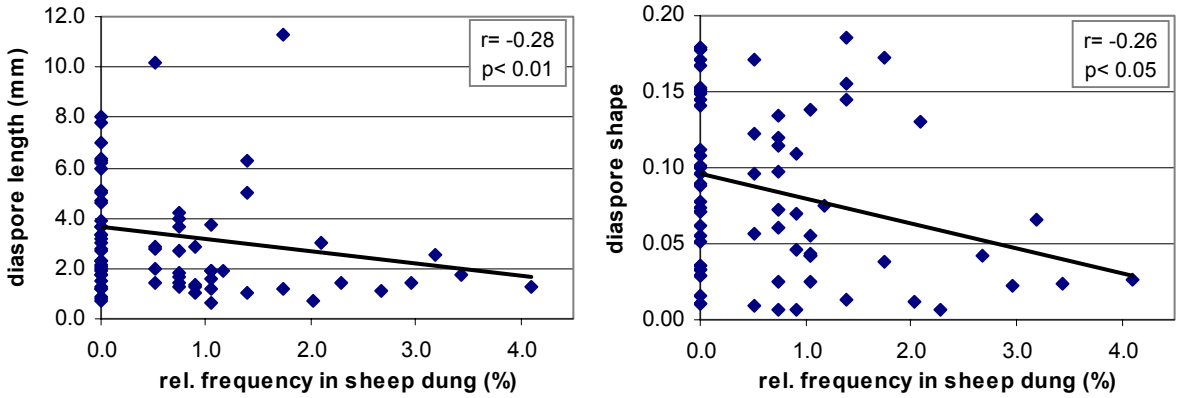


Fig. 5.8a: Significant correlations between the relative frequency of plant species in dung samples of grazing sheep (study I, KUTZKE 1998) with different diaspore and plant traits as well as phenological data.

Spearman's rank correlation coefficient; data include species fructifying but not present in the dung samples (0% relative frequency); data concerning germination in dung were square-root transformed; diaspore length: n=74; diaspore shape: n=74.

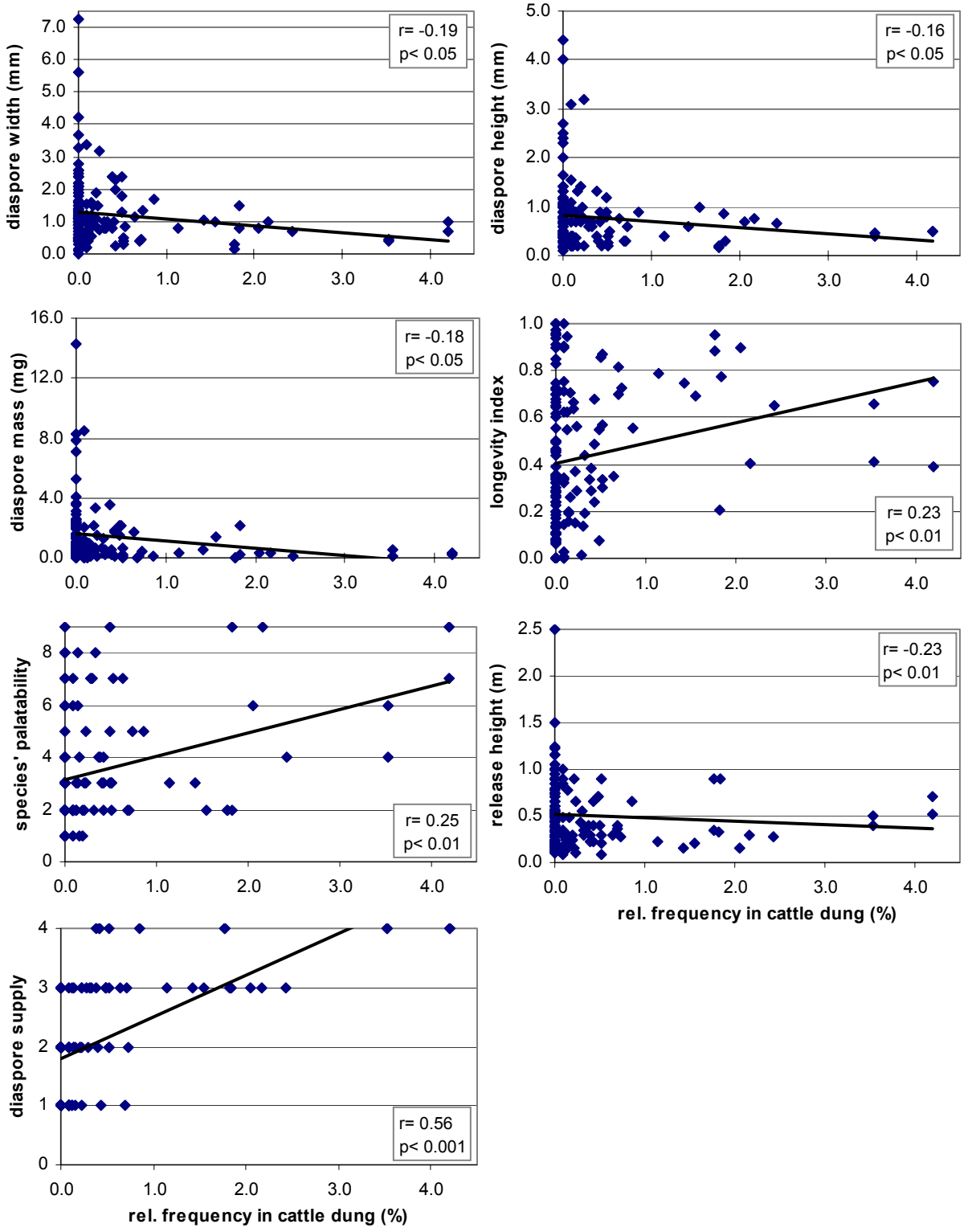


Fig. 5.8b: Significant correlations between the relative frequency of plant species in dung samples of grazing cattle (study II, STENDER 1996) with different diaspore and plant traits as well as phenological data.

Spearman's rank correlation coefficient; data include species fructifying but not present in the dung samples (0% relative frequency); data concerning germination in dung were square-root transformed; diaspore width: n=145, diaspore mass: n=147, seed bank longevity index: n=135, release height: n=147, diaspore supply: n=147, species' palatability: n=136.

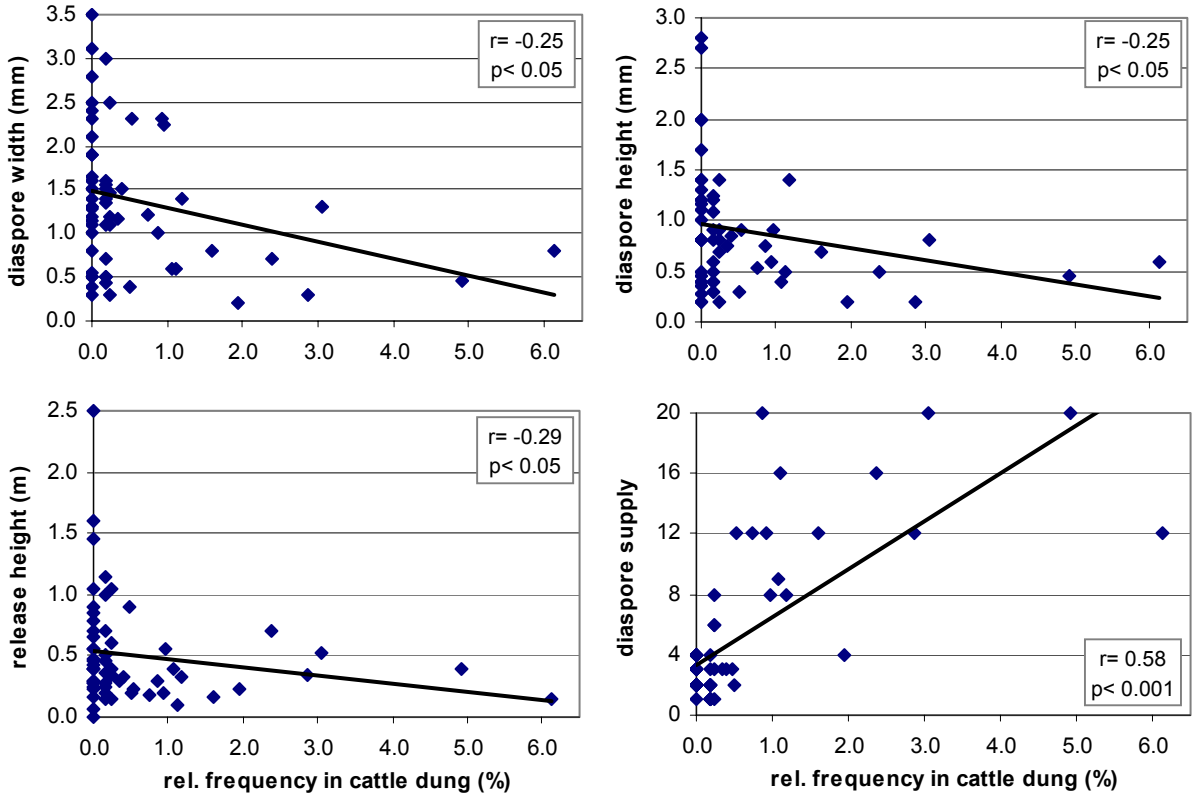


Fig. 5.8c: Significant correlations between the relative frequency of plant species in dung samples of grazing cattle (study III, SCHÖNFELDER 1996) with different diaspore and plant traits as well as phenological data.

Spearman's rank correlation coefficient; data include species fructifying but not present in the dung samples (0% relative frequency); data concerning germination in dung were square-root transformed; diaspore width: n=61, diaspore height: n=61, release height n=61, diaspore supply: n=61.

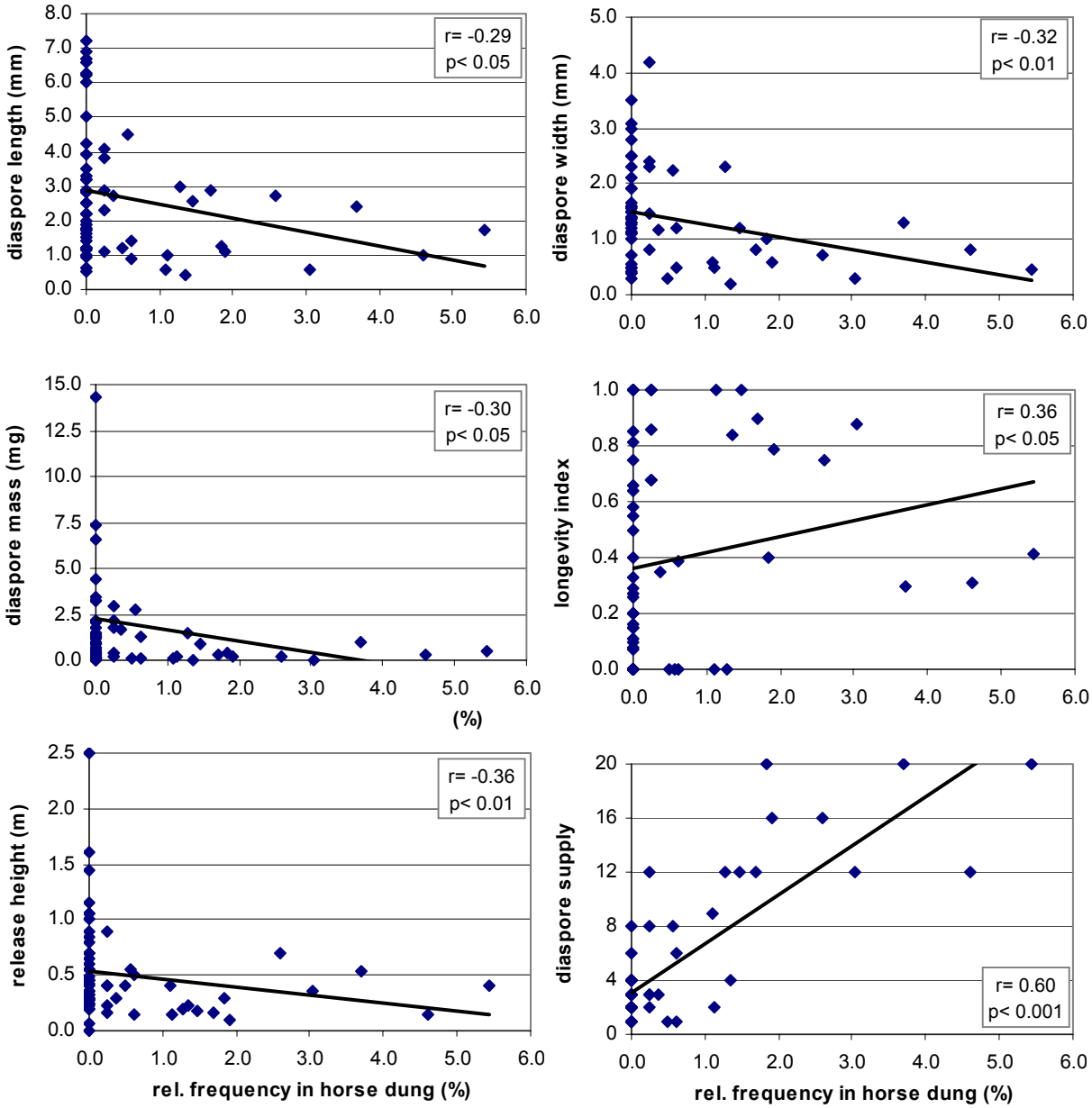


Fig. 5.8d: Significant correlations between the relative frequency of plant species in dung samples of grazing horses (study III, SCHÖNFELDER 1998) with different diaspore and plant traits as well as phenological data.

Spearman's rank correlation coefficient, data include species fructifying but not present in the dung samples (0% relative frequency); data concerning germination in dung were square-root transformed; significant correlation of relative frequency with diaspore height is not illustrated; diaspore length: n= 61, diaspore height: n=61, diaspore mass: n=59, seed bank longevity index: n=44, release height: n=61, diaspore supply: n=61.

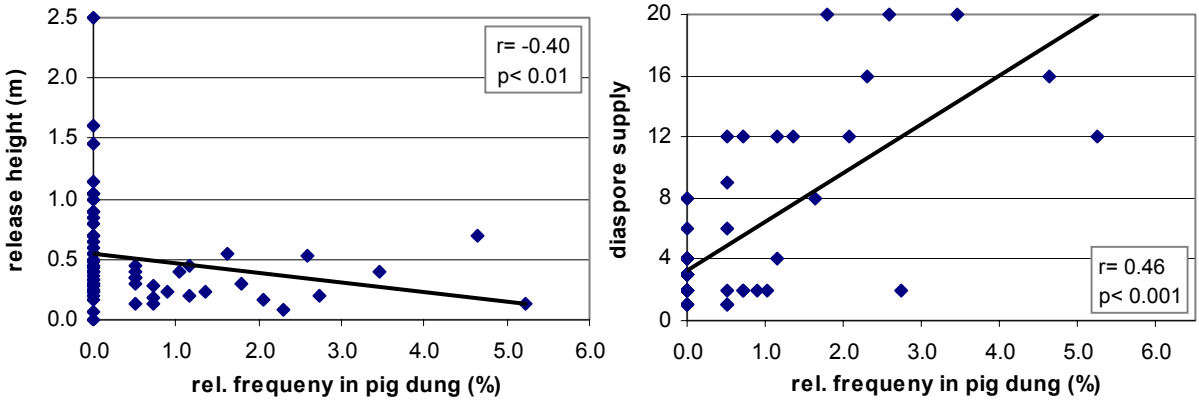


Fig. 5.8e: Significant correlations between the relative frequency of plant species in dung samples of grazing pigs (study III, SCHÖNFELDER 1998) with different diaspore and plant traits as well as phenological data.

Spearman's rank correlation coefficient, data include species fructifying but not present in the dung samples (0% relative frequency); data concerning germination in dung were square-root transformed; release height: n=61, diaspore supply: n=61.

Discrimination of grouped data of diaspore content in the dung of grazing herbivores by traits of the diaspore, plant and site (pasture)

Separating data into two groups according to the presence or absence of fructifying plant species in the collected dung, best results were obtained when either all explaining variables were included in the analysis (Table 5.9, 1) or those which were independent from direct measurements on the diaspore (3). As already indicated by Spearman's rank correlations (Table 5.10, Fig. 5.8), diaspore supply was one of the discriminating variables except for study 1 (sheep).

The occurrence of plant species in the dung of sheep was explained by the seed bank longevity index, which however, led to an unsatisfying separation of the groups (eigenvalue 0.058) and a low reliability of the function.

Groups of diaspore frequency in cattle and horse dung of study 3 were discriminated by diaspore supply and seed bank longevity index, whereas release height was the second discriminating variable in study 2. Only the occurrence of plant species in pig dung was explained by diaspore supply alone. The highest classification accuracy (>80%) was obtained when discriminating plant species' occurrence in horse and pig dung. However, eigenvalues and Wilks's λ indicate that the quality of discrimination by the chosen variables is higher for species occurrence in horse dung than in pig dung. Any reduction of the number of explaining variables led to an identical or even poorer result of data discrimination for all studies.

When plant species' frequency in the collected dung was subdivided into three groups (Table 5.9 continued), reliability of discrimination became poorer in relation to the binary data-classification according to the presence and absence of plant species in dung. However, the higher eigenvalues

indicate a better between-group separation by the discriminant functions. For all studies, diaspore supply was chosen as a discriminating variable when including either all explaining variables (1) or only diaspore supply, release height, seed bank longevity index and species palatability (3) in the analysis. When additional variables were chosen as predictors, such as seed bank longevity index, release height and/or diaspore width in study 2 and 3, diaspore supply had the highest relative importance. Restricting explaining variables to morphological diaspore traits (length, width, height, shape) and diaspore mass led for no studies to any result (2).

Table 5.9 Results of stepwise discriminant analysis for data grouped with regard to the presence of plant species in the dung of different grazing animals.

1) all explaining variables included; see Table 5.3);
2) only morphological diaspore traits and diaspore mass included;
3) only seed bank longevity index, release height, diaspore supply and palatability included;

figures separated by "/" relate to the different functions of multiple discriminant analysis; n.s.: not significant; *: significant at p<0.05; **: significant at p<0.01; ***: significant at p<0.001.

	CLASSIFICATION INTO TWO GROUPS (presence/absence in dung)				
	sheep	**cattle**	**cattle**	**horse**	**pig**
	study 1	study 2	study 3		
discriminating variables; standardized canonical discriminant function coefficients in brackets	1) longevity index (1) 2) - 3) =1)	1) diaspore supply (0.897); release height (-0.534) 2) diaspore width (1) 3) diaspore supply (0.887); release height (-0.501)	1) diaspore supply (0.842), longevity index (0.585) 2) - 3) =1)	1) diaspore supply (0.922), longevity index (0.558) 2) - 3) =1)	1) diaspore supply (1) 2) - 3) =1)
	quality of discrimination				
eigenvalue	1) 0.058 2) - 3) =1)	1) 0.369 2) 0.038 3) 0.363	1) 0.639 2) - 3) =1)	1) 1.211 2) - 3) =1)	1) 0.857 2) 0.142 3) =1)
Wilks's λ	1) 0.954 (n.s.) 2) - 3) =1)	1) 0.730 *** 2) 0.936 * 3) 0.734 ***	1) 0.614 *** 2) - 3) =1)	1) 0.452 *** 2) - 3) =1)	1) 0.538 *** 2) 0.876 (n.s.) 3) =1)
correctness of classification	1) 58.3% 2) - 3) =1)	1) 71.2% 2) 56.3% 3) 70.5%	1) 75.0% 2) - 3) =1)	1) 81.8% 2) - 3) =1)	1) 80.3% 2) - 3) 3) =1)

Table 5.9 continued Results of stepwise discriminant analysis for data grouped with regard to the presence of plant species in the dung of different grazing animals.

1) all explaining variables included; see Table 5.3);
2) only morphological diaspore traits and diaspore mass included;
3) only seed bank longevity index, release height, diaspore supply and palatability included;

figures separated by "/" relate to the different functions of multiple discriminant analysis; n.s.: not significant; *: significant at p<0.05; **: significant at p<0.01; ***: significant at p<0.001.

	CLASSIFICATION INTO THREE GROUPS (rel. frequency in dung 0% / 0-1% / >1%)				
	sheep	**cattle**	**cattle**	**horse**	**pig**
	study 1	study 2	study 3		
discriminating variables; standardized canonical discriminant function coefficients in brackets	1) diaspore supply (1) 2) - 3) =1)	1) diaspore supply (0.927 / 0.135), longevity index (0.470 / 0.369), release height (-0.229 / 0.998) 2) - 3) diaspore supply (0.934 / -0.389), longevity index (0.525 / 0.864)	1) diaspore supply (1) 2) - 3) =1)	1) diaspore supply (0.985 / 0.043), diaspore width (-0.221 / 0.864), longevity index (0.360 / 0.781) 2) - 3) diaspore supply (0.999 / -0.253), longevity index (0.433 / 0.928)	1) diaspore supply (1) 2) - 3) =1
	quality of discrimination				
eigenvalue	1) 0.206 2) - 3) =1)	1) 0.704 / 0.032 2) - 3) 0.675 / 0.001	1) 1.084 2) - 3) =1)	1) 3.173 / 0.241 2) - 3) 3.036 / 0.065	1) 1.775 2) - 3) =1)
Wilks's λ	1) 0.830 ** 2) - 3) =1)	1) 0.569 *** 2) - 3) 0.597 ***	1) 0.480 *** 2) - 3) =1	1) 0.193 *** 2) - 3) 0.233 ***	1) 0.360 *** 2) - 3) =1)
correctness of classification	1) 41.9 % 2) - 3) =1)	1) 60.0% 2) - 3) 63.7%	1) 60.7% 2) - 3) =1)	1) 77.3% 2) - 3) 72.7%	1) 75.4% 2) - 3) =1)

Discussion

Correlations of diaspore survival from different feeding experiments with diaspore and plant traits

All approaches intending to explain endozoochorous survival capacity of plant species with diaspore and plant traits regarding diaspore morphology, mass, specific gravity, various measurements of the seed coat, diaspore longevity in the seed bank and plant species' palatability gave quite heterogeneous results. Table 5.10 gives an overview over the Spearman's rank correlation coefficients obtained when correlating survival rates after gut passage with the chosen diaspore and plant traits. At least one of the different morphological diaspore traits (**diaspore length, width, height and shape**) correlated significantly with the survival rates of half of the

evaluated feeding experiments. All these correlations indicate a negative relationship between morphological diaspore traits and survival capacity. **Specific gravity** was positively correlated with diaspore survival determined in feeding experiment II (Göttingen) and the aggregated feeding experiments including several published studies concerning endozoochorous dispersal. All significant correlations with traits concerning diaspore morphology and specific gravity found in the present studies are supported by previous feeding experiments (e.g. RUSSI et al. 1992; GARDENER et al. 1993a; GHASSALI et al. 1998) and they are reasonable from the point of animal physiology. Selective particle retention by the reticulo-omasal orifice is size dependant. Bigger particles are therefore retained longer in the rumen than smaller ones (e.g. POPPI et al. 1980; ULYATT et al. 1986; KASKE & ENGELHARDT 1990). A longer retention time increases the detrimental effects of digestive processes and therefore affects diaspore survival negatively (ATKESON et al. 1934; SIMAO NETO & JONES 1987). Because the size of particles too large to pass the reticulo-omasal orifice has to be reduced and rumination has the primary function of reducing the particle size (ULYATT et al. 1986), bigger diaspores have also a higher risk of being damaged or killed by mastication. For sheep it was shown that particles longer than 1 to 2 mm have a low probability of leaving the reticulo-rumen (POPPI et al. 1980; WELCH 1982). Threshold particle size in cattle is 1.5 to 2.0 times higher than that of sheep (ULYATT et al. 1986), therefore diaspore survival is usually lower when passing the alimentary tract of sheep.

Specific gravity of ingested feed is supposed to have an even more important role than diaspore size with respect to selective particle retention (EHLE & STERN 1986). As a three-phase compartment – a gas-cap above a floating raft or mat on a liquid pool – the ruminal raft is composed of more recently ingested forage particles that are not dense enough to sediment. Rumination and digestion reduce particle size, resulting in particles that are more dense (VAN SOEST 1994). Only particles with a high specific gravity sink and are most likely to be selectively transported through the reticulo-omasal orifice (DESBORDES & WELCH 1984; KASKE & ENGELHARDT 1990)[*]. Therefore, particles with a high specific gravity may leave the reticulo-rumen faster, which explains the positive correlations with survival capacity found in the evaluated studies (see also GARDENER et al. 1993a).

Floating capacity of a diaspore, which is important for the residence time in the ruminal raft, is not only a function of specific gravity but also of diaspore shape. The latter is quantified by the extent to which it differs from a sphere; the minimum value of zero indicates a perfect sphere (BAKKER et al. 1996). Diaspores with a spherical shape sink faster and are, therefore, able to leave the reticulo-rumen faster than longish diaspores. This explains a negative correlation between diaspore survival and diaspore shape (see also GÖKBULAK 2002). Furthermore, a spherical shape may be more advantageous to escape from the ruminal raft.

[*] Particles with a very high specific gravity (~>1.5 g/ml) are again retained longer in the reticulo-rumen (EHLE & STERN 1986; KASKE & ENGELHARDT 1990). However, feed particles do not reach a specific gravity >1.5 g/ml (EVANS et al. 1973).

Table 5.10 Correlations (Spearman's rank correlation coefficient) between plant species' survival capacity when passing the digestion tract of different animals and various traits of the plant, diaspore and site (pasture).

**: significant at p<0.01; *: significant at p<0.05; n.s.: not significant.

	feeding experiment 1 (IGER)		**feeding experiment 2 (Göttingen)**		**incl. feeding experiments from literature**	
	sheep	**cattle**	**sheep**	**cattle**	**sheep**	**cattle**
diaspore length	0.28 n.s. (n=14)	0.50 n.s. (n=14)	-0.41 n.s. (n=12)	-0.54 n.s. (n=12)	**-0.29** * (n=63)	-0.17 n.s. (n=64)
diaspore width	0.30 n.s. (n=14)	0.42 n.s. (n=14)	0.06 n.s. (n=12)	-0.15 n.s. (n=12)	-0.08 n.s. (n=63)	0.03 n.s. (n=64)
diaspore height	0.23 n.s. (n=14)	0.39 n.s. (n=14)	0.13 n.s. (n=12)	0.43 n.s. (n=12)	0.09 n.s. (n=63)	0.02 n.s. (n=64)
diaspore shape	-0.01 n.s. (n=14)	-0.01 n.s. (n=14)	**-0.58** * (n=12)	**-0.66** * (n=12)	**-0.25** * (n=63)	-0.15 n.s. (n=64)
diaspore mass	0.12 n.s. (n=14)	0.32 n.s. (n=14)	0.48 n.s. (n=12)	0.28 n.s. (n=12)	0.04 n.s. (n=63)	0.10 n.s. (n=64)
specific gravity	0.22 n.s. (n=14)	0.23 n.s. (n=14)	**0.58** * (n=12)	**0.59** * (n=12)	**0.38** ** (n=62)	**0.34** ** (n=64)
total thickness of the seed	-0.03 n.s. (n=12)	0.17 n.s. (n=12)	-	-	-	-
thickness of the seed coat without cell lumen	-0.07 n.s. (n=12)	0.15 n.s. (n=12)	-	-	-	-
total thickness of all phenol-containing	-0.15 n.s. (n=12)	0.05 n.s. (n=12)	-	-	-	-
thickness of the thickest phenol-	-0.15 n.s. (n=12)	0.04 n.s. (n=12)	-	-	-	-
longevity index	-0.13 n.s. (n=14)	-0.12 n.s. (n=14)	-0.03 n.s. (n=12)	-0.14 n.s. (n=12)	0.09 n.s. (n=61)	0.12 n.s. (n=63)
palatability	0.24 n.s. (n=12)	0.42 n.s. (n=12)	**0.66** * (n=12)	**0.58** * (n=12)	0.13 n.s. (n=55)	**0.37** ** (n=56)
release height	-	-	-	-	-	-
diaspore supply	-	-	-	-	-	-

Table 5.10 **continued** Correlations (Spearman's rank correlation coefficient) between plant species' survival capacity when passing the digestion tract of different animals and various traits of the plant, diaspore and site (pasture).

***: significant at p<0.001; **: significant at p<0.01; *: significant at p<0.05; n.s.: not significant.

	dung collected from grazing livestock				
	sheep study I	**cattle study II**	**cattle study III**	**horse study III**	**pig study III**
diaspore length	**-0.28** ** (n=74)	-0.11 n.s. (n=146)	-0.19 n.s. (n=61)	**-0.29** * (n=61)	-0.17 n.s. (n=61)
diaspore width	-0.06 n.s. (n=74)	**-0.19** * (n=145)	**-0.25** * (n=61)	**-0.32** ** (n=61)	-0.20 n.s. (n=61)
diaspore height	-0.03 n.s. (n=74)	**-0.16** * (n=146)	**-0.25** * (n=61)	**-0.34** ** (n=61)	-0.17 n.s. (n=61)
diaspore shape	**-0.26** * (n=74)	0.09 n.s. (n=146)	0.01 n.s. (n=61)	0.06 n.s. (n=61)	-0.04 n.s. (n=61)
diaspore mass	-0.16 n.s. (n=74)	**-0.18** * (n=147)	-0.22 n.s. (n=59)	**-0.30** * (n=59)	-0.13 n.s. (n=59)
specific gravity	-	-	-	-	-
total thickness of the seed coat	-	-	-	-	-
thickness of the seed coat without cell lumen	-	-	-	-	-
total thickness of all phenol-containing layers	-	-	-	-	-
thickness of the thickest phenol-containing	-	-	-	-	-
longevity index	0.16 n.s. (n=72)	**0.23** ** (n=135)	0.29 n.s. (n=44)	**0.36** * (n=44)	0.20 n.s. (n=44)
palatability	0.13 n.s. (n=72)	**0.25** ** (n=136)	-0.01 n.s. (n=53)	0.10 n.s. (n=53)	0.21 n.s. (n=53)
release height	-0.03 n.s. (n=74)	**-0.23** ** (n=147)	**-0.29** * (n=61)	**-0.36** ** (n=61)	**-0.40** ** (n=61)
diaspore supply	0.07 n.s. (n=72)	**0.56** *** (n=147)	**0.57** *** (n=61)	**0.60** *** (n=61)	**0.46** *** (n=61)

More conspicuous than the significant correlations found are the numerous non-significant correlations between plant and diaspore traits and endozoochorous survival capacity. Again, several other studies confirm this lack of any correlation between the above mentioned diaspore traits and species survival capacity when passing ruminants gut (e.g. LEHRER & TISDALE 1956; SIMAO NETO et al. 1987) or at least exceptions from the general trend (THOMSON et al. 1990).

DOUCETTE et al. (2001) even found that retention times of different diaspores were not influenced by diaspore size and density. Accordingly it was shown that particles larger than the cited critical size (see above) can to a certain degree pass the reticulo-omasal orifice despite selective particle retention (ULYATT et al. 1986; LECHNER-DOLL et al. 1991). DESWYSEN & ELLIS (1990) further point to difficulties of measuring a threshold value for the size of particles leaving the rumen in absolute terms because of biological randomness in the passage of particles.

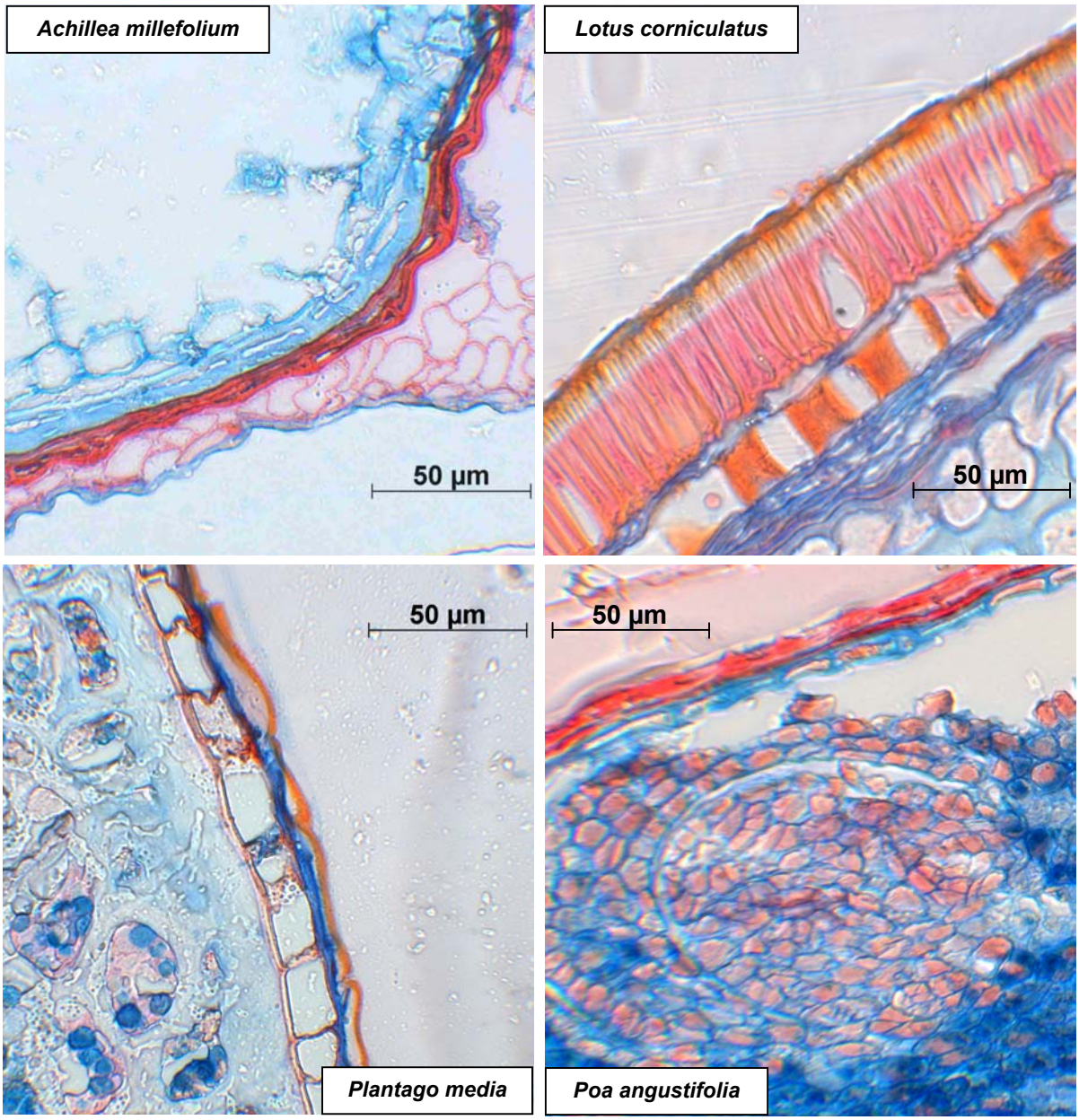


Fig. 5.9 Seed coat structure of *Achillea millefolium*, *Lotus corniculatus*, *Plantago media* and *Poa angustifolia* after staining with safranin-astrablue.

None of the **seed coat characteristics,** which only have been evaluated for the species fed in feeding experiment I (IGER), showed a significant correlation with species survival after the passage of the alimentary tract of sheep or cattle. The lack of any correlation was not surprising, since species with a high or intermediate survival, as *Achillea millefolium*, *Plantago media* or *Poa angustifolia* (Fig. 5.9), had a very thin seed coat, whereas e.g. *Lotus corniculatus* (Fig. 5.9) or *Salvia pratensis* (Fig. 5.2) survived the passage of the digestive tract in very low quantities despite a relatively thick seed coat (Table 5.6). Interestingly, thickness of the seed coat of both legumes, *Anthyllis vulneraria* (Fig. 5.2) and *Lotus corniculatus* (Fig. 5.9), was quite similar, although *Anthyllis* proved to have a higher content of phenols and lignins. However, survival capacity of both species differed more than it would be expected from these differences in the content of phenols and lignins. Consequently, the selected traits of the structures surrounding the embryo and endosperm alone cannot explain the observed differences in endozoochorous survival capacity of the fed species.

For legumes, WERKER (1997) and BASKIN et al. (2000) postulate that the palisade layer is responsible for physical dormancy and therefore impermeable for liquids. Besides a tight package of the palisade cells, the cell walls must be impregnated with water-resistant substances (WERKER 1980/81), which are primarily located in the outer ends of the palisade cells (VAN STADEN et al. 1989). These impregnated cell walls were visible in the seed coats of both legumes (s. Fig. 5.2 and 5.9) and can, therefore, not be the reason for differences in survival capacity. However, the palisade layer of *Lotus corniculatus* (Fig. 5.9) showed little breaches which could not be observed in the one of *Anthyllis vulneraria* (Fig. 5.2). These breaches may have caused a higher permeability of the seed coat of *Lotus corniculatus* and may, therefore, be one explanation for the its lower endozoochorous survival capacity. However, other structures of the seed coat which are possibly relevant for endozoochorous survival capacity were not considered in the present study. The permeability of legume seeds, for example, is caused by the rupture of the strophiole (BASKIN et al. 2000). Hence, differences in the structure of the strophiole may influence survival capacity. In other species, differences in the structure and permeability of the micropyle may cause differences with respect to the tolerance of the microbial and chemical attacks in the digestive tract. In conclusion, single seed coat characteristics are too divers in their functions to be related to survival capacity.

Survival rates documented in feeding experiment II (Göttingen) as well as survival obtained by the aggregated feeding experiments showed significant correlations with plant **species' palatability**. However, correlations with feeding experiment II (Göttingen) are affected by the share of legumes, which are usually classified as palatable (except of *Genista* ssp., *Ononis* ssp.; KLOTZ et al. 2002) and show intermediate or high survival rates, depending on the proportion of hard seeds. However, a high palatability is also attributed to legume populations with low proportions of hard seeds (e.g. *Lotus corniculatus* in feeding experiment I), which makes the existence of an adaptation of legumes to endozoochorous dispersal dubious. Together with uncertainties concerning the reliability of existing classification systems of palatability (BRIEMLE 2002, see below) it is therefore not possible to conclude from the results obtained by the different feeding experiments whether more palatable species are adapted to endozoochorous dispersal by herbivores.

Prediction of survival capacity of plant species by diaspore and plant species traits

Following single correlations between endozoochorous dispersal potential and the different plant or diaspore traits as well as the results of previous studies and knowledge of animal physiology, there are various diaspore traits facilitating an unharmed passage through the gut (e.g. hardseededness, small size, high specific gravity). Nevertheless, except for species producing a certain amount of hard seeds, it was not possible to decide by these traits whether a species has an endozoochorous dispersal potential or not. Neither spearman's rank correlations nor discriminant analyses gave unique results with regard to an explanation of survival capacity of plant species by diaspore and plant species traits. A discrimination of species survival capacity obtained in single studies by the evaluated traits resulted even in a high classification accuracy (e.g. Table 5.7). Nevertheless, it was not possible to use any of the discriminant functions for the prediction of the results of another feeding experiment. Although single variables, as plant species' palatability, were used as one of several variables for the discrimination of different data sets, the resulting discriminant functions differed a lot. These results reflect the variety of relationships documented in literature.

Several reasons may explain the varying results. Because the number of plant species fed in single feeding experiments is usually restricted to few species, species choice influences statistical analyses with single diaspore or plant traits very much. The negative relationships between survival capacity and size parameters of the diaspore, for example, which have been found in several of the illustrated studies as well as in literature (SIMAO NETO et al. 1987; RUSSI et al. 1992; GHASSALI et al. 1998), were not found in feeding experiment I (IGER). This was attributed on the one hand to the high survival of *Anthyllis vulneraria* and – for diaspores digested by cattle – *Sanguisorba minor* with its big diaspores. On the other hand, *Jasione montana* should have good chances to pass the digestive tract quickly and unharmed due to its minute diaspores, but no diaspores germinated after passing the ruminants gut and even not after a mere exposure of undigested *Jasione*-diaspores on dung (control). This species has also never been found in any other study on endozoochorous dispersal (source: DIASPORUS, BONN et al. 2000). However, when less heterogeneous (only Poaceae or legumes, e.g. GHASSALI et al. 1998) or bigger data sets (as shown by the aggregation of various feeding experiments) are used for the calculation of correlations of morphological diaspore traits with survival capacity, significant relationships can be found.

Nevertheless, such "exceptions" of the general trend, as illustrated by *Anthyllis, Sanguisorba* or *Jasione*, show that an accurate assessment of endozoochorous dispersal potential by diaspore or plant traits will always remain uncertain. Survival is influenced by a variety of traits and there is no monocausal explanation, as it often has been treated in previous studies. The existence of a certain trait favouring an unharmed passage through the alimentary tract may compensate for properties disadvantageous for a save passage. On the other hand, traits promoting endozoochorous dispersal may be insignificant because of other disadvantageous traits. A small diaspore size, for example, may be useless with regard to a quick passage when the diaspore has a very low specific gravity (KASKE & ENGELHARDT 1990), because very small particles with a high buoyancy may be retained in the rumen mat and therefore have a lower probability to escape from the reticulo-rumen (FAICHNEY 1986; OKINE et al. 1998). On the other hand, KIRK & COURTNEY (1972)

attributed the higher survival of dehusked and scarified *Avena fatua*-diaspores in relation to intact ones to their smaller diaspores size, compensating the higher sensitivity of the unprotected caryopsis. Survival promoting traits, such as a high specific gravity or a spherical shape, may be ineffective when a diaspore is very big and therefore chewed more intensively or retained very long in the reticulo-rumen. A hard seed coat may to a certain degree compensate for a big diaspore size, whereas traits promoting a fast passage of the gut may compensate for a thin seed coat. In summary, there are not single traits but trait combinations that influence a diaspores passage through the digestive tract and the variety of trait combinations is great.

In order to assess the relative importance of one single trait for diaspore survival and not the interaction of several varying traits, future studies should concentrate on a species choice, where only one single trait is varied (e.g. NORTON et al. 1989 for hardseededness).

Correspondence of correlates from different feeding experiments with germination in dung samples excreted by grazing herbivores

Corresponding to the results of the evaluated feeding experiments, weak but significant negative correlations with various traits concerning diaspore size and shape were obtained for most of the dung samples collected from grazing livestock (Table 5.10). Because larger diaspores are usually heavier, negative correlations found for diaspore mass and plant species' frequency in the dung of grazing cattle and horses may be interpreted as another measure for the negative relationship between diaspore size and survival capacity (see also THOMSON et al. 1990). Whereas correlations with survival rates of the feeding experiments may be influenced by the species set fed, the evaluation of dung samples with a spontaneous feed intake confirm the existence of a negative relationship between survival capacity and morphological parameters of diaspores (see also PAKEMAN et al. 2002). However, as already shown for the feeding experiments, the relation to one of the size- or shape-related traits is not obligatory.

With regard to further morphological diaspore attributes, MALO & SUÁREZ (1995) showed that diaspores possessing special adaptations for dispersal, such as plumes or awns, were less frequent in herbivore dung. The existence of such appendages may be disadvantageous to escape the ruminal raft. In the present study the proportion of these species in the different dung samples was similar or only slightly lower than on the pasture (Fig. 5.7). Diaspore quantities were, however, in general much lower than those of species with unspecialised or minute diaspores. Species with a high diaspore supply on the pasture but only rare or even no germination in the exposed dung often had awned, spiny or plumed diaspores (e.g. *Agrimonia eupatoria, Brachypodium pinnatum, Carlina acaulis, Leontodon hispidus, Scabiosa columbaria*). On the other hand, there were also other species missing in the dung samples despite a high diaspore supply (e.g. *Dianthus carthusianorum*, *Teucrium chamaedrys*). Hence, the presence or absence of diaspore appendages seems to be also no reliable criterion to assess endozoochorous survival capacity.

Contrary to the results obtained by the feeding experiments, where no significant correlations with the seed bank longevity index could be found, this trait was correlated with the "dung flora" of

grazing cattle and horses and was also used as one of the discriminating variables predicting survival capacity. Correlations between the presence of species in dung samples of grazing herbivores and the seed bank longevity index have already been found by PAKEMAN et al. (2002). Diaspore longevity in the seed bank is affected by morphological diaspore traits as well as by the resistance of the seed or fruit coat against physical and chemical attacks (THOMPSON et al. 1993, 1998; BAKKER et al. 1996, 2000). The correlations found may, therefore, reflect the relations between diaspore morphology and endozoochorous survival capacity, because the longevity index also shows negative correlations with diaspore size and shape (THOMPSON et al. 1993). Already BAKKER et al. (1996) postulated that diaspore characteristics, such as weight, size and shape, may be linked to both, dispersal in space and time. Although measurements of the thickness and phenol content of the seed coat showed no correlations with survival capacity in the present study (see above), certain characteristics of the seed or fruit coat may help to withstand the attacks in the soil as well in the digestive tract of herbivores. In both environments, diaspores are exposed to cellulolytic enzymes, although they occur in the soil under aerobic conditions (PAUL & CLARKE 1996). Already JANZEN (1984) pointed to the fact that a seed coats ability to resist digestion is also a valuable trait to persist in the soil. Nevertheless, as indicated by the fact that only two of five studies and none of the feeding experiments showed significant correlations between survival capacity and the longevity index, the relevance of this trait seems to be not really high. This is also indicated by low correlation indeces. However, the lack of any significant correlation between survival capacity of the fed species and the longevity index may be also be influenced by the low number of studies for the calculation of the longevity index of several plant species, leading to a low reliability of the calculated index.

No significant correlation was obtained for germination rates in the dung of pigs and seed bank longevity index, although NEUGEBAUER (2004) showed that species germinating in pig dung and those germinating from the diaspore bank of the grazed pasture correlated significantly. Pigs ingest diaspores of the seed bank together with the soil they usually incorporate (BRIEDERMANN 1986). Species present in the seed bank simply have to be regarded as an additional, below-ground diaspore supply.

Apart from traits of the diaspore different traits of the plant as well as of the grazed site obviously influence endozoochorous dispersal during grazing. Except for sheep, plant species frequency in the collected dung samples showed negative correlations with the release height of the diaspores and positive correlations with diaspore supply. Negative correlations between a diaspores releasing height and its frequency in dung are the result of grazing habits. Cattle, for instance, feed in a height of 8 to 20 cm, whereas higher parts of the sward are not eaten or at least less frequent (JOHNSTONE-WALLACE 1956 in STENDER et al. 1997). Similar feeding habits have to be supposed for other herbivores. To a certain degree, a high diaspore supply seems to compensate unfavourable diaspore traits with regard to an unharmed passage through the digestive tract, because more diaspores are ingested. The obtained strong correlations implicate that these traits of the parent plant or the grazed pasture, both influencing diaspore intake, are more important for

endozoochorous dispersal than diaspore traits influencing survival during the passage of the digestive tract. In the discriminant analyses, however, only diaspore supply was regularly used for discriminating plant species with respect to their presence in herbivore dung and it proved for nearly all data sets to be the most important discriminating variable. Contrary to these results, PAKEMAN et al. (2002) even found negative correlations between the abundance of species on the pasture and in the dung of herbivores, whereas MALO & SUÁREZ (1995) concluded from studies of dung from different herbivores that the seed content in herbivore dung depends heavily on pasture phenology and "that dispersal via excrements depends mainly on the seeds present in each period".

At the landscape level, diaspore supply was also found of being important for long distance dispersal by other dispersal modes. As supposed for endozoochory, a high diaspore production may compensate for a lower wind dispersal potential (TACKENBERG 2001; TACKENBERG et al. 2003). Rare long-distance dispersal events by wind become more probable when more diaspores are available. The frequency of plant species was also an important parameter for epizoochory (FISCHER et al. 1996). In summary, diaspore supply seems to have a very high importance for long-distance dispersal by various dispersal vectors. Species with a high diaspore production at the population level will always have a higher probability of being dispersed over long distances, regardless by which dispersal vector. The assessment of diaspore production is, however, complicated by the fact that it is a very variable trait and may vary over more than five orders of magnitude between different populations of the same species (SERA & SERY 2004). It therefore seems not to be a suitable trait for the assessment of dispersal potential at the species level (TACKENBERG et al. 2003).

Despite the correlations found between diaspore supply and diaspore frequency in dung, endozoochorous dispersal by different herbivores was also selective (Fig. 5.10, see also MALO & SUÁREZ 1995). Selectivity may be the result of differences in the capacity to survive the passage of the alimentary tract, as it was demonstrated for several plant species in the evaluated feeding experiments. Selectivity during grazing may be another factor causing differences in plant species occurrence in dung. JONES & BUNCH (1999), for example, found diaspores of the dominant grass species on the pasture only rarely in the faeces of grazing cattle (see also WELCH 1985), which was attributed to the low palatability of this species. In contrast, other, less dominant species were more frequent in the collected dung than it would have been expected from their occurrence in pasture vegetation. For some species with an extremely high frequency in rabbit dung, such as *Urtica dioica*, PAKEMAN et al. (1998, 2002) hypothesize selective grazing or the occurrence of frugivory rather than a simple removal of diaspores during eating. Similar observations were made by ECKERT (1995) as well as KIEFER (1999): after fruit ripening, grazing sheep were eating preferentially the infructescence of *Urtica dioica*, which resulted in extraordinary high seedling numbers in exposed dung samples (<50% of all seedlings germinating in the collected dung samples, KIEFER 1999). In the evaluated studies plant species palatability correlated only in one study significantly with germination rates in the collected dung (s. Tab. 5.10). However, data used concerning species palatability actually indicate the forage value of a plant species and mostly

relate to the vegetative plant (KLAPP 1971, BRIEMLE 2002). Until now, only few studies actually tried to assess plant species selection by different ruminants on the pasture, because it depends on several, extremely variable factors such as state of physiological development, frequency at the pasture, composition and frequency of the remaining feed at the pasture, pollution by excrements or fungi, stocking rate etc. (KLAPP 1971; JONES et al. 1991; PORZIG & SAMBRAUS 1991; FORBES 1995). However, an accurate assessment of the relevance of this trait for endozoochorous dispersal requires more detailed studies on feed selection during grazing.

Feeding experiments versus dung collected from grazing livestock to assess endozoochorous dispersal potential

The number of diaspores produced on the population level proved to be of a high importance for the presence of diaspores in the dung of grazing herbivores, whereas morphological diaspore traits only had a minor relevance. Therefore, it may be questioned whether survival capacity of plant species determined by feeding experiments or other experimental approaches are transferable to the landscape level, that is diaspore dispersal within a pasture or between different grazed habitats.

A comparison of a plant species' survival capacity evaluated in feeding experiments and diaspores germinating in the dung of grazing sheep and cattle in relation to the diaspores produced on the pasture (Fig. 5.10) shows corresponding as well as differing results. In correspondence to their high survival in feeding experiments several plant species showed a high frequency in the dung of grazing herbivores in relation to diaspore supply. Other species with low or no surviving diaspores after feeding often were also rare or missing in herbivore dung despite an intermediate or even high diaspore supply. However, for each study, there are discrepancies between both methodological approaches, too. For legumes (Fig. 5.10, *Trifolium campestre, Anthyllis vulneraria, Lotus corniculatus* and *Onobrychis sativa (*sheep), *Trifolium repens, Lotus corniculatus* and *Trifolium pratense* (cattle II)) these differing results may be explained by varying proportions of hard seeds of diaspores fed and those swallowed on the pasture, because hardseededness strongly determines endozoochorous survival capacity (chap. 4, SIMAO NETO & JONES 1987; NORTON et al. 1989). The proportion of hard seeds may be extremely variable between different populations of the same species (NORTON et al. 1989) and may even vary within the same population from year to year (JONES & BUNCH 1988). For legumes and other plant families with physical dormancy a determination of the proportion of hard diaspores would therefore be the most accurate way to estimate endozoochorous dispersal potential (chap. 4, see also GARDENER et al. 1993b).

Contradicting results of both experimental approaches were also obtained for several species of other plant families (Fig. 5.10). Diaspores of *Lolium perenne*, for example, did not germinate in the dung of grazing sheep despite a high survival rate in a feeding experiment and an intermediate diaspore supply on the pasture. In contrast, *Thymus pulegioides* germinated in the dung of grazing sheep in higher quantities than it would have been expected from its survival after feeding. In the dung of cattle I several species were rare or even missing although they showed

high survival rates in feeding experiments and produced numerous diaspores on the pasture (e.g. *Phleum pratense, Silene alba, Plantago lanceolata, Geranium pusillum, Rumex acetosa*). The same accounts for most plant species of the third study (cattle II). Similar to legumes this may be due to differences between single populations of a species with regard to the capacity to pass the ruminant gut. Different sensitivities of diaspores of the same plant species from different origins were also documented for treatments simulating single steps of ruminant digestion (see chap. 4, Fig. 4.17). Endozoochorous survival capacity evaluated by feeding experiments may be, therefore, not transferable to another population of the respective species. A simultaneous determination of endozoochorous dispersal potential of plant species from the same population and year by a feeding experiment and by collecting dung from grazing herbivores might show whether there is a higher correspondence of both experimental approaches.

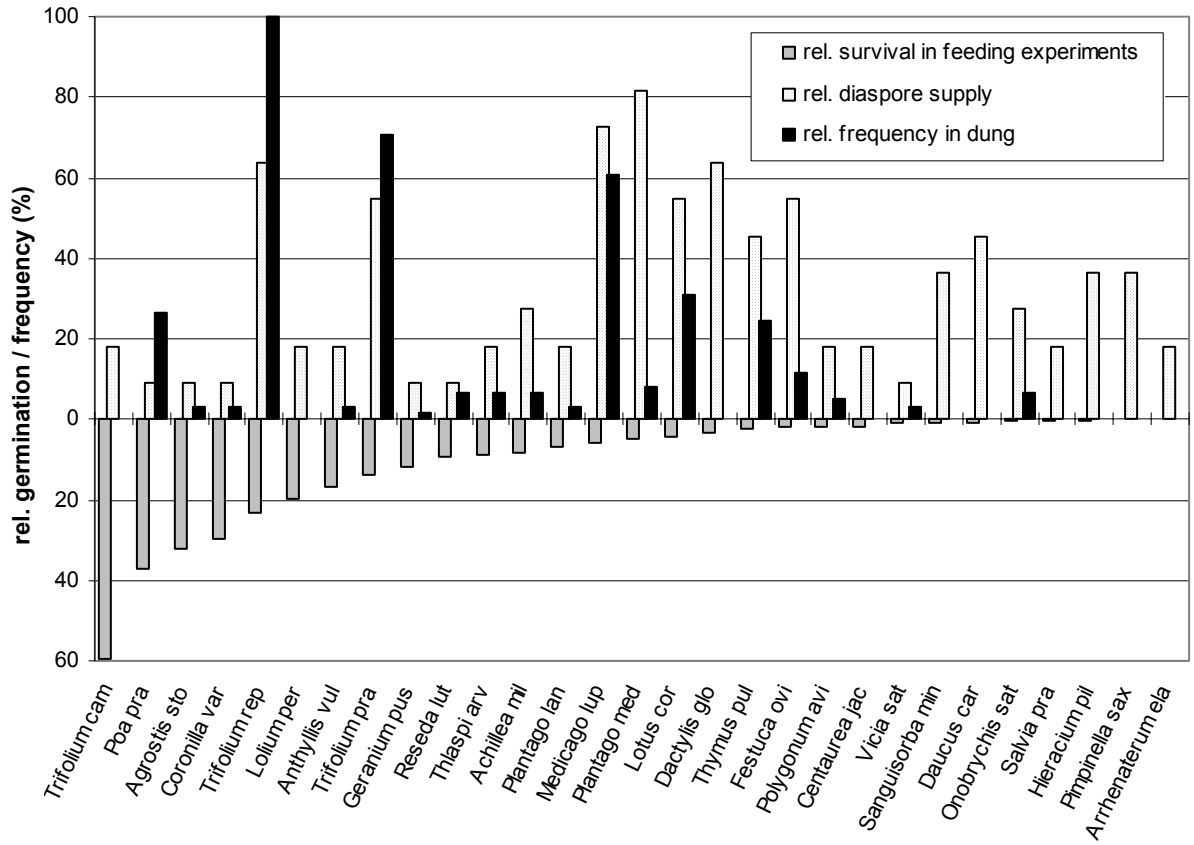
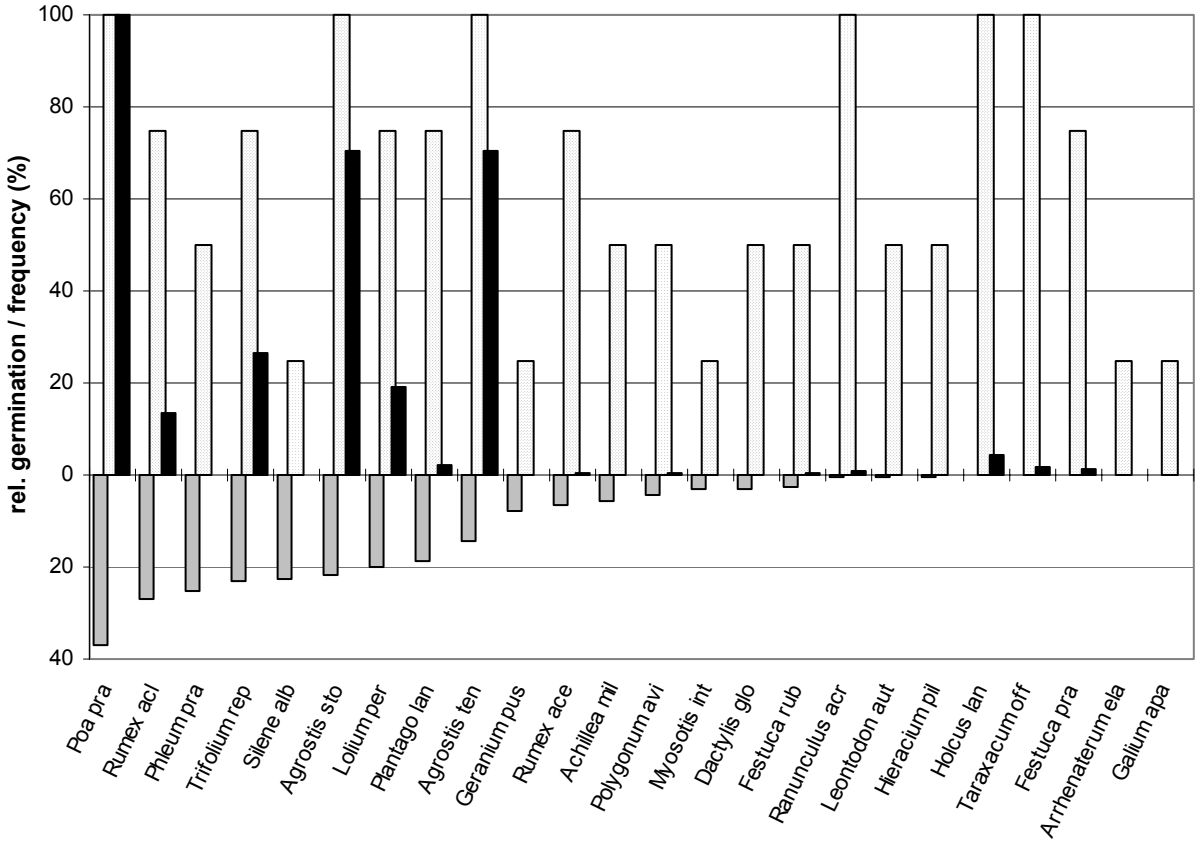


Fig. 5.10a Comparison of relative survival of different plant species after diaspore feeding to sheep and the frequency of the corresponding species in the dung of grazing sheep in relation to diaspore supply on the pasture.

Relative germination in feeding experiment relates to the number of viable diaspores fed; relative diaspore supply relates to the highest diaspore supply of a species on the pasture; relative frequency in dung relates to the species with the highest frequency in dung collected from grazing sheep. Data are illustrated only for those species, for which survival capacity has been evaluated by feeding experiments and which were documented as fructifying on the pasture.



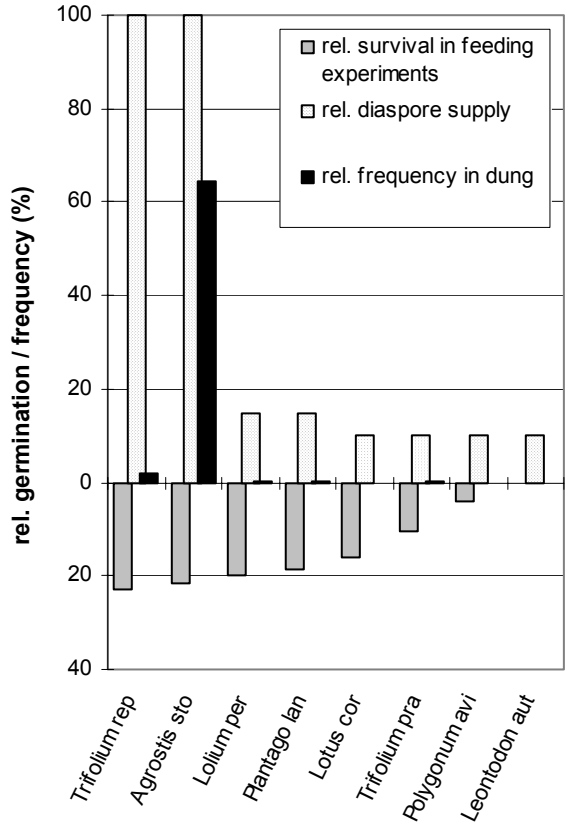


Fig. 5.10b Comparison of relative survival of different plant species after diaspore feeding to cattle and the frequency of the corresponding species in the dung of grazing cattle in relation to diaspore supply on the pasture (cattle I and II relate to different studies).

Relative germination in feeding experiment relates to the number of viable diaspores fed; relative diaspore supply relates to the highest diaspore supply of a species on the pasture; relative frequency in dung relates to the species with the highest frequency in dung collected from grazing cattle. Data are illustrated only for those species, for which survival capacity has been evaluated by feeding experiments and which were documented as fructifying on the pasture.

However, there may exist further reasons for a lacking correspondence of the results of feeding experiments and germinating diaspores in collected dung samples of grazing herbivores in relation to diaspore supply. Selectivity during grazing (see above) may be responsible for deviations from the dung flora expected from diaspore supply on a pasture or survival capacity determined by feeding experiments. An exemplary assessment of feed selection on the pasture and diaspores germinating in the dung of grazing herbivores might show to which extent the proportion of diaspores in the dung is influenced by feed selection. Furthermore, a low diaspore content of a plant species in the dung of grazing herbivores despite a high diaspore supply may also result from a low initial germination rate of the diaspores (see also NEUGEBAUER 2004).

Because the determination of endozoochorous dispersal potential focuses ecological relevant dispersal processes – dispersal processes within or between grazed habitats – the causes for the observed discrepancies between the results of feeding experiments and germinating diaspores in the dung of grazing herbivores in relation to diaspore supply should be examined in future studies.

Chapter 6 Conclusion and perspectives

Large omni- and herbivores are assumed to have been one of the most important dispersal vectors for herbaceous plant species in the postglacial time, especially with respect to long distances (e.g. RIDLEY 1930; JANZEN 1981; PAKEMAN 2001). With the beginning settlement of man in the Neolithic age, the relevance of natural dispersal processes, as those by wild animals, decreased in favour of different agricultural practices affecting the dispersability of plants (BONN & POSCHLOD 1998a,b). Taking into account that domestic livestock was extremely widespread in the traditional man-made landscape, as it was herded in forests, on arable fields, grasslands as well as on all kinds of marginal habitats, as heath- and peatland, animals probably represented again one of the most important local and regional dispersal vectors (POSCHLOD & BONN 1998). Consequently, animals played an outstanding role for the dispersal of plant species for millennia. In present times, when the loss of dispersal processes between habitats is discussed as one important reason for the decrease of plant species diversity, the consideration of endozoochorous dispersal is therefore crucial in the context of conservation biology and restoration ecology in man-made landscapes.

Information concerning the determinants of endozoochorous dispersability of diaspores are, however, quite rudimentary. Only recently, massive deficits concerning the present knowledge on long-distance diaspore dispersal in general were also emphasized by CAIN et al. (2000) as well as HIGGINS et al. (2003). Since long-distance dispersal events are nearly impossible to measure (SILVERTOWN & LOVETT-DOUST 1993), dispersal relevant parameters or traits allowing a prediction or modelling of dispersal processes are required (HIGGINS et al. 2003).

A comprehensive database on diaspore dispersal with data on documented dispersal modes and dispersal related diaspore and plant traits has to be judged as an extremely useful tool for conservation biology, because it allows to predict the consequences of changes in land use or other measurements in the context of nature conservation for certain plant species or all species of a community (chap. 3; BONN et al. 2000). This could be illustrated by exemplary applications of the database »Diasporus«. However, the quality of a database as well as of the developed forecasts or models depends on the reliability of its data sets. Standardised data on dispersal relevant morphological traits as well as aggregated dispersal relevant traits or “indicator parameters” (e.g. terminal velocity, attachment capacity) as basic elements of such applications are still very scarce or even missing completely (see also TACKENBERG et al. 2003). This accounts for endozoochory as well as for all other dispersal modes relevant for long-distance dispersal. Therefore, a reliable comprehensive database on diaspore dispersal has to be regarded as a final step in the assessment of a plant species dispersability, which is preceded by the assessment of single dispersal modes by standardized methods and plant or diaspore traits, respectively.

The results of the present study revealed that the use of a standardized method (simulated chewing (2x) followed by an immersion in 0.1 M HCl (8 hours)) is more appropriate to assess endozoochorous dispersal potential than different traits of the diaspore (size, mass, specific gravity, seed coat thickness etc.), although a further validation of the method is recommended. The standardized method is based on differences in the sensitivty of diaspores to a combination of mechanical and chemical strain, as it occurs during feed comminution and digestion by a ruminant. It does, however, not take into account diaspore-specific differences in retention time or differences in the frequency of rumination, which are relevant for the survival of gut passage and which are often attributed to morphological traits of the diaspore. Corresponding correlations between survival rates after in vivo-digestion and different morphological traits of the diaspore were also found for some of the feeding experiments evaluated in the present study. On the one hand, it is clear that a method neglecting these differences during digestion will always have a limited accuracy in simulating in vivo-survival capacity. On the other hand, intraspecific variations between individual ruminants concerning chewing behavior and feed digestion (ULYATT et al. 1986; KASKE & ENGELHARDT 1990) as well as differences in survival capacity within the same plant species justify a rough assessment of endozoochorous dispersal potential.

Furthermore, the actual importance of single traits for diaspore retention and survival is not yet clear: although there was a trend to negative correlations of survival rates with size-related diaspore traits or diaspore shape (according to THOMPSON et al. 1993) and positive correlations to specific gravity, there were also several differences between the studies evaluated. Varying results concerning the role of single traits of the diaspore for endozoochorous dispersability were also found in previous studies (e.g. THOMSON et al. 1990; DOUCETTE et al. 2001). The valuation of the influence of single diaspore traits is, however, in most experiments complicated by the choice of the studied plant species. Because diaspores of different species usually differ with regard to several diaspore traits (e.g. shape, size, mass), a distinction between the influence of single traits is usually not possible. In order to assess the relative importance of one single trait for diaspore survival when passing the digestive tract, it therefore would be elucidating to concentrate on a species choice, where only one single trait is varied (e.g. NORTON et al. 1989).

Diaspore survival after standardized treatments simulating certain parts of the digestive system differed among different populations of the same plant species, indicating intraspecific differences in endozoochorous survival capacity. Therefore, it seems advisable to include different populations of a plant species in the analysis in order to estimate the intraspecific variability of endozoochorous dispersal potential of a species. Future studies should also be interested in the causes of observed differences, e.g. differences in the history of land-use. A comparison of endozoochorous survival capacity of plant species from long-term grazed and mown grasslands, for instance, would be interesting with respect to the evolution of a higher tolerance of species from the grazed habitat to pass the ruminant digestive system.

In contrast to the varying results concerning the influence of traits of the diaspore or parent plant on endozoochorous survival capacity, diaspore supply proved for nearly all studies to be an important parameter for the frequency of a species in the dung of grazing herbivores. A high

diaspore supply on a grazed habitat may compensate to a certain degree for a low endozoochorous survival capacity because of a higher diaspore input. However, diaspore supply seems not to be a suitable trait for the assessment of dispersal potential at the species level, because it is extremely site-specific and variable (TACKENBERG et al. 2003; SERA & SERY 2004). Because it was shown that diaspore supply influences other modes of dispersal in the same way (e.g. epizoochory: FISCHER et al. 1996; anemochory: TACKENBERG 2001; TACKENBERG et al. 2003), the estimation of diaspore production in a certain habitat may serve as an alternative possibility for a rough assessment of long-distance dispersal potential of a species or all species of a community, relating to the specific site.

Comparing the results of feeding experiments and the frequency of the respective species in the dung of grazing herbivores in relation to diaspore supply on the pastures showed for part of the evaluated species contradicting results. Different sensitivities of diaspores of the same plant species from different populations when passing the digestive tract, selectivity during grazing or differences concerning the initial germination rate of the ingested diaspores were supposed as possible reasons for these discrepancies. In future studies, it would be advisable to test these hypothesis because feeding experiments or other standardized methods are only tools to assess ecological relevant dispersal processes – endozoochorous dispersal by grazing herbivores. In order to guarantee a transferability of experimentally assessed data, deviations between both approaches (experimental approaches and dung collected from grazing herbivores) have to be understood.

Chapter 7 Summary

Dispersal is one of the fundamental processes in the life cycle of each plant. Dispersal affects the distribution of species, population dynamics, intraspecific competition and the level of gene flow. On the community level dispersal is related to coexistence and may limit species richness, diversity and dynamics of plant communities. However, until recently it remained more or less disregarded in plant ecology, because studies on diaspore dispersal often were restricted in documenting single dispersal events or in classifying the different modes of dispersal, deducing the dispersal mode of a species from diaspore morphology. Gradual differences in dispersal potential as well as the manifold dispersal processes in our cultural landscape were usually not considered. However, a risk assessment for plant species in the context of conservation biology and restoration ecology requires not only a knowledge concerning the loss or changes of dispersal processes in our landscape but also adequate methods or "tools" to predict the dispersability of a certain plant species by various dispersal vectors or to detect dispersal limitations. Such methods or tools aiming at a quantification of a species' dispersal potential may be developed by the collection of existing data concerning dispersal modes and distances as well as dispersal related diaspore and plant traits in a data base, by deriving the dispersal potential from dispersal relevant diaspore and plant attributes or from the assessment of dispersal via standardized methodological approaches. This thesis focuses on the three mentioned approaches as well as the change of dispersal processes in the Central European landscape.

In chapter 2 it is illustrated that during the evolution of the Central European landscape and especially since the settlement of man there was a permanent change of processes affecting the dispersability of plants. The highest diversity of dispersal processes combined with a high diversity of land use practices existed in the traditional man-made landscape. Most of these processes changed or became completely lost in the actual man-made landscape. Due to the improvement of seed cleaning, for example, a lot of weeds became extinct which were spread with contaminated seed in former times. Changing harvest methods were responsible for the selection of weeds which ripe later and produce light diaspores. Traditional manure as well as the big variety of additional materials used as fertilizer contained huge amounts of diaspores whereas today animal slurry with low contents of diaspores or mineral fertilizer are used. The abandonment of artificial flooding, which favoured the migration of species in meadows of mountain and floodplain regions, is regarded as one reason for the increasing losses of grassland species. Herded and transhumant domestic livestock which was probably the most important dispersal vector in the Central European man-made landscape dramatically decreased or vanished locally completely.

Whereas in the traditional man-made landscape all habitats were more or less connected due to alternating management practices or grazing today most habitats are isolated. Important dispersal processes of modern times, e.g. those caused by traffic, cannot substitute dispersal vectors and processes of the traditional man-made landscape. The consideration of dispersal processes or vectors should therefore be one basic element in the restoration of habitats. If there is no possibility to restore traditional dispersal processes these processes have to be simulated or replaced by other ones which may also include the artificial reintroduction of species.

In chapter 3 »Diasporus«, a database on dispersal characteristics, is presented as one tool for the risk assessment of plant species. »Diasporus« contains data on documented dispersal modes as well as standardised species related traits relevant for seed or diaspore dispersal.

Basic steps in the development of the database included the classification of different modes of dispersal and the classification of plant or diaspore traits relevant for dispersal between populations as well as to suitable but so far unoccupied habitats (”long-distance dispersal”). Most existing classifications show a more or less strict combination of the dispersal mode and morphological characters of the diaspore or plant. In contrast, the classification of »Diasporus« is exclusively based on different dispersal vectors, while diaspore morphology is viewed as one of several factors affecting the dispersal potential of plant species. Besides single dispersal relevant traits of the adult plant or the diaspore (time and period of dispersal, diaspore production per plant, releasing height, occurrence of xero- or hygrochasy, diaspore size, shape, weight, specific gravity and further morphological traits, such as structure of the diaspore surface or a hard seed coat), indicator parameters, aggregating various morphological traits, were considered in the classification of dispersal relevant traits. Standard methods for the calculation of these indicator parameters (terminal velocity, buoyancy, attachment capacity and digestion resistance) are proposed.

The database consists of several linked tables, containing the traits of the species studied, the methods used in a dispersal experiment or in determining a certain dispersal relevant trait, and the references for the citation of the literature. The structure of »Diasporus« allows all possible combinations of data included in the reference-, methods- and species-tables. Some examples for the application of the database in the context of viability analysis or risk assessment in plants are given, illustrating the advantages of a comprehensive database on diaspore dispersal.

In chapter 4 and 5, two approaches for the assessment of dispersal potential are presented. Because diaspore dispersal via passing the digestive system of ruminants is an important dispersal mechanism in natural as well as cultural landscapes, these two approaches were exemplified for endozoochory.

The first study (chapter 4) concerns different experimental approaches to assess endozoochorous dispersal potential. Since feeding experiments are very elaborate and time consuming, they have been conducted only with few plant species. Therefore, various approaches were tested in order to find an alternative, preferably easy and quick method to assess

endozoochorous dispersal potential. The approaches were related to the results of a feeding experiment with 14 plant species fed to cattle and sheep. The effects of mastication, microbial and chemical digestion as the basic digestion steps in the ruminant digestive system were simulated by three different approaches: in vitro-digestion (rumen fluid followed by acidified pepsin), simulated chewing followed by an immersion in acid (HCl), and imbibition in anoxic water followed by an immersion in acid (HCl). Although survival after in vitro-digestion showed the best correlations with survival after in vivo-digestion, it was rejected as a standard method because of the continuous need of rumen fluid. Mastication, simulated by mechanical stress via an iron stick, followed by an immersion in 0.1 M HCl over 8 hours proved to correlate also strongly with the results of the feeding experiment, especially with the survival rates after passing the digestive tract of sheep. Because sheep provide a more conservative estimate of a plant species survival capacity when passing the ruminants gut, this method is recommended as a suitable standard method to estimate endozoochorous dispersal potential. For legumes and other species with physical dormancy a determination of the proportion of hard diaspores is proposed as an alternative method to assess endozoochorous dispersal potential. For both approaches, it seems advisable to consider intraspecific differences in the tolerance to survive the passage through the digestive tract. With respect to the grade of specification of endozoochorous dispersal potential a four graded classification with the lowest grade for those species incapable to survive the passage of the digestion tract is recommended.

In chapter 5 attributes of the diaspore and plant were tested for their qualification to predict endozoochorous dispersal potential. Three approaches were used to assess endozoochorous dispersal potential via plant and diaspore traits: 1. two different feeding experiments, 2. data from literature combined with the data of the two feeding experiments in order to obtain a greater number of species and 3. dung samples from grazing herbivores in relation to diaspore supply on the pasture. Diaspore and plant traits tested for their suitability to predict endozoochorous dispersal potential were diaspore morphology (length, width, height, shape), diaspore mass, specific gravity, seed bank longevity index, plant species' palatability and attributes of the seed or fruit coat (thickness, phenol content). Release height and diaspore supply were included as further variables for species germinating in the dung of grazing herbivores or fructifying on the pasture.

Correlations between endozoochorous dispersal potential and the different traits evaluated were quite variable concerning the different approaches. In general, negative correlations were obtained between one of the morphological diaspore traits and relative frequency in herbivore dung. Nearly all data sets of germinated diaspores in the dung of grazing animals showed highest significant correlations with traits relating not to the dispersal unit: release height (negative correlation) and diaspore supply on the pasture (positive correlation). The relevance of diaspore supply was also reflected by discriminant analysis, where this trait proved to be for nearly all data sets the most important discriminating variable. Although various other variables affect endozoochorous dispersal potential, this indicates that the most important variable influencing endozoochory is diaspore supply, which is not only species specific but also influenced by site

conditions and, therefore, extremely variable. Using discriminant analysis, however, no transferable statistic models to predict endozoochorous dispersal potential by diaspore or plant traits could be identified.

Comparing endozoochorous survival capacity of a plant species assessed by feeding experiments and the frequency of the corresponding species in the dung of grazing herbivores in relation to the diaspore supply showed contradicting results for several plant species. Different sensitivities of diaspores of the same plant species from different populations when passing the digestive tract, selectivity during grazing or differences concerning the initial germination rate of the ingested diaspores were supposed as possible reasons for these discrepancies.

In chapter 6 conclusions are drawn and perspectives for future studies concerning endozoochorous dispersal are shown.

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